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Editor

ROY WALDO MINER

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*SECTION OF BIOLOGY**

CHEMICAL PROPERTIES OF THE TETRAZOLIUM SALTS**

By John B. Rust

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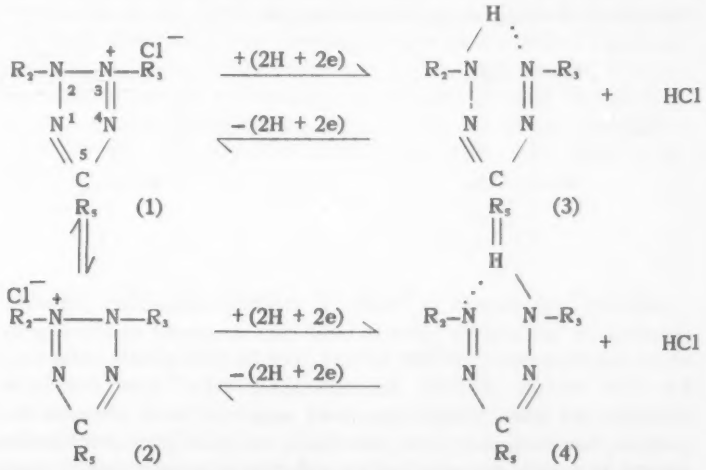
Tetrazolium salts[1,2,3] are representative of a unique class of compounds which are finding increasing use in biological and chemical research due to their fortunate combination of desirable properties. They are quaternary ammonium salts and, as such, possess a high degree of water solubility. The water solubility, of course, varies considerably and depends upon the nature and properties of the substituent groups, but is usually sufficiently great to allow the salts to be used in water solution.

The normally colorless, or pale-colored tetrazolium salts possess the property of being easily transformed into intensely colored, water-insoluble formazans by reduction. This property is opposite to the general behavior of such dyestuffs as the indophenols, azines, oxazines, indigoid, and flavins, which yield colorless compounds, or leuco bases on reduction.

The transformation occurs through a rupture of the tetrazole ring:



*The Section of Physics and Chemistry held a meeting on February 1, 1955 at which a paper entitled "New Developments in Photographic Chemistry" was presented by W. H. Vinton. No abstract of this paper has been received.

The Section of Geology and Mineralogy held a meeting on February 7, 1955 at which a paper entitled "Macroevolution and Some Moot Aspects of the Origin of Resemblances" was presented by Alexander Petrunkevitch. No abstract of this paper has been received.

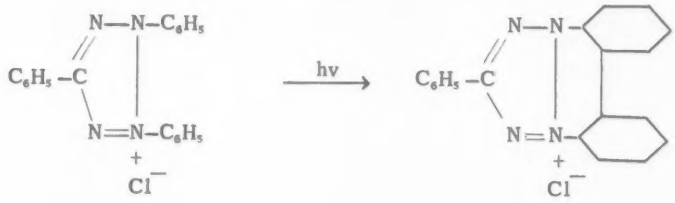
**This paper, illustrated with lantern slides, was the first of five papers in a symposium on Recent Advances in the Biological Application of Tetrazolium Salts presented at a meeting of the Section on February 14, 1955.

A tautomeric relationship between structures (1) and (2), which may be explained in terms of cationotrophy between the two forms, renders positions 2 and 3, on the tetrazole ring, equivalent, as does also tautomerism due to chelate hydrogen bonding between structures (3)and (4) in the formazan. This has been experimentally confirmed for a large number of substituted tetrazolium salts.

Under strongly reducing conditions, reduction of the formazan can continue to yield colorless compounds, although this does not apparently take place in biological systems.

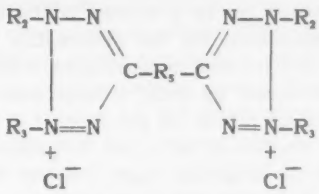
In general, tetrazolium salts are photosensitive, the gross change observable with visible light being the acquisition of a yellow color. Although visible light causes yellowing, ultraviolet light of short-wave length brings about reduction to the formazan in aqueous solution. The formazans are also light-sensitive. Upon exposure to visible light, formazan will undergo a color change from red[4] to a yellow modification. However, if this yellow modification is then placed in the dark, the color gradually returns to its original red. The phenomenon is due to a cis-trans isomerization.

Irradiation with ultraviolet light for long periods, however, produces more deep-seated changes. In the case of triphenyltetrazolium chloride,[5] intense irradiation of its ethanol solution for about 14 hours results in the formation of 2,3(2,2′-biphenylene)-5-phenyl-2H-tetrazolium chloride. This chemical change is an irreversible one.



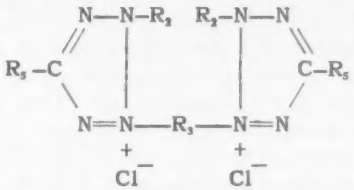
Although the change in color of triphenyltetrazolium chloride on reduction is sufficiently great to make this compound emminently useful as an indicator, there was felt to be a need for still greater color contrast. For this reason, in 1947, bis-tetrazolium salts[6] were introduced by Pannone and Rust. These compounds appeared to be adequate for the purpose for which they were developed, and biological use has demonstrated that they possess certain selective properties which were not at first anticipated.

There are two types of bis-tetrazolium salts possible, which possess somewhat different properties. Those of least interest at the present time are formed by linkage through the 5-positions.

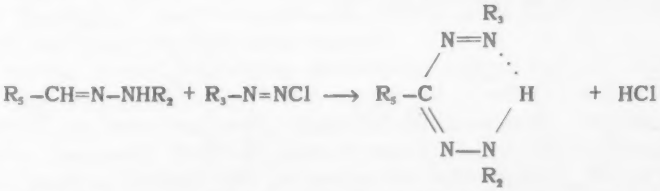


The linking group R_5 can be alkyl or aryl. Although not of great interest or use at the present time, these salts may possess certain attractive properties which will be brought out only when they have become experimentally available and when their characteristics have been ascertained.

The other type of bis-tetrazolium salt that has proved of great utility is represented by neotetrazolium and blue tetrazolium.



Formazan synthesis. Aldehyde-hydrazones are coupled with diazonium salts to yield formazans. The coupling occurs on the active carbon of the aldehyde under controlled conditions and in the presence of acid-binding agents which remove the acid formed as rapidly as it is liberated. In general, any aldehyde-hydrazone[1] may be used, but only the aldehyde-phenylhydrazones, or substituted phenylhydrazones, have been fully investigated.



The aldehydes used have been both aliphatic and aromatic and the hydrazine bases have been phenylhydrazine and the substituted phenylhydrazines. In like manner, the diazonium salts have been obtained from various diazotizable aromatic amines.

The conditions for coupling are subject to considerable variation depending upon the properties of the compounds to be reacted. Several general conditions may be ennunciated, however, which must be observed for the proper conduct of the reaction.

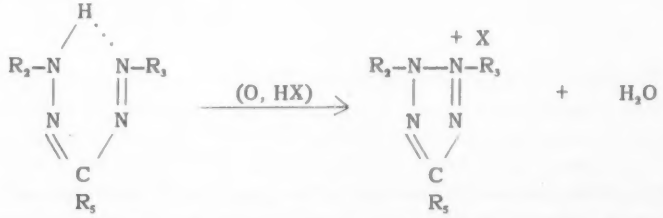
The reaction must be carried out in a solvent which is capable of dissolving each of the components, but not necessarily the formazan which is formed. Finding this solvent is sometimes difficult, but the difficulty can usually be overcome by using a large amount of an acceptable solvent such as ethanol, dioxan, or pyridine.

The reaction should be conducted at a low temperature to avoid premature decomposition of the diazonium salt. Usually a temperature of 0° to −5° C. is satisfactory, although temperatures as high as 18° to 20° C. may be used if the diazonium salt is somewhat stable or if the solution is dilute.

It is very important that the acid liberated during the reaction be neutralized as it is formed. It is equally important, however, that no great concentration of strong base be present during the entire reaction, since such a state of affairs would contribute to the rapid decomposition of the diazonium salt before it could react with the aldehyde-hydrazone, thus resulting in a lowered yield of formazan. The two usual procedures are to employ either an excess of a weak base, such as pyridine or sodium acetate, or to add a solution of a strong base slowly and simultaneously with the addition of the diazonium salt. When pyridine is employed, it also acts as a strong solvent for the reactants. When sodium acetate is used, a large excess of alcohol must be employed. Alcohol is usually used in the case where a strong base, such as sodium hydroxide, is employed.

The isolation and purification of the formazans presents no exceptional problems and is carried out in the ordinary manner of recrystallization, precipitation, or adsorption and desorption.

Tetrazolium salt synthesis. To form the tetrazolium salt, a formazan is simultaneously cyclicized and quaternized by oxidation in the presence of an acid. This may be accomplished in a variety of ways, all of which are essentially an oxidation reaction.



The oxidants which have been employed with success are the alkyl nitrites, sodium nitrite, lead tetracetate,[7] the alkyl hypochlorites, manganese dioxide, yellow mercuric oxide, hydrogen peroxide, nitric acid, and similar oxidants. A large variety of acids can be used to produce the desired tetrazolium salt directly, or an intermediate salt may first be prepared and the desired compound obtained from it by

double decomposition.

As in the case of the formazans, there are several general conditions for the formation of the tetrazolium salts which are important. In the first place, it is necessary to provide a solvent which must have the property of a solvent for the tetrazolium salt which is formed. It is highly desirable that this solvent also be a solvent for the formazan, although it does not have to have great solvent power for this role. It is necessary to employ a sufficient volume of this solvent to moderate the oxidation reaction which is exothermic. The usual solvents which are employed are ethanol, isopropanol, n-pentanol, and chloroform.

There may be some limitations to the oxidation reaction, especially where there are oxygen-labile substituent groups on the formazan as, for instance, in the case of the sugar formazans.[8]

The isolation of the tetrazolium salt is accomplished by first filtering off inorganic salts if they are present, then evaporating off the solvent. If sufficiently soluble, the tetrazolium salt is taken up in water, separated from tarry products which are formed during the oxidation reaction, decolorized with activated carbon, and crystallized. Purification is accomplished by recrystallization from water or from other suitable solvent after additional treatment with carbon. If the tetrazolium salt is only sparingly soluble in water, a similar procedure is used, except that some other suitable solvent, such as ethanol, is employed. At times it is highly useful to utilize the complexes that some tetrazolium salts form with solvents, notably chloroform.

Reduction. The tetrazolium salts have a low redox potential in the neighborhood of −0.08 volts,[15] which makes them especially useful as reduction indicators in biological systems and chemical investigations. Apparently no systematic research has been conducted on the effect of structure or substituent groups on this potential,[9] although such a line of investigation could be particularly fruitful, especially when coupled with the biological effect of the reagents.

Several general, qualitative observations may be made on the conditions favoring reduction, however. The pH of water solutions of the tetrazolium salts vary greatly depending upon their purity, structure, and anion component. Therefore, general practice is to maintain the pH of the solution constant with an appropriate buffer system. The ease of reduction then increases with increasing pH. In some cases, color formation occurs spontaneously at pH 9 to 10. Furthermore, reduction occurs more readily at elevated temperatures. With mild reducing agents, such as reducing sugars,[10] reducing steroids,[11] and the like, it is necessary to maintain a high pH in the neighborhood of 12, where most of the tetrazolium salt is probably in the form of its hydroxide. In biological systems, a pH of over 6 is desirable.

Besides chemical and biological reduction, tetrazolium salts are

converted into formazan under the influence of high energy particles and radiation, such as energetic electrons, ultraviolet light, α-particles, X rays, γ-rays and the like. Exposure of triphenyltetrazolium chloride in 1 per cent aqueous solution to ultraviolet light[12] gives triphenyl formazan. By exposing a gelled agar solution to cathode radiation,[13] reduction takes place along the path of the radiation.

The photochemical reduction of triphenyltetrazolium chloride in aqueous solution depends upon the wave length, and the amount of reduction is proportional to the intensity and the time of exposure.[14] Wave lengths longer than 3,650 Å have little or no effect; whereas those between 3,650 Å and 2,536 Å are effective. With X radiation, about 32,000 r. is required for the first visual observation of a red precipitate.

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SECTION OF BIOLOGY

STUDIES OF THE METABOLISM OF FIBROBLAST CELLS IN TISSUE CULTURE UTILIZING NEOTETRAZOLIUM*

By William Antopol, George H. Fried, and Helen Kodza

Laboratories of the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, New York, N. Y.

The tetrazolium salts have been widely used as a tool in the study of cell metabolism. The ability of these comparatively colorless agents to accept hydrogen from a suitable donor, with the formation of an insoluble colored formazan at the site of reduction, provides a tool for histochemical demonstration of vital activity. [1, 2, 3, 4, 5] Using these compounds as indicators, and with the proper substrates, it has been possible to indicate the presence and probable location of a number of respiratory enzymes in living tissue. [6]

A number of investigators [1, 2, 7] have demonstrated that neotetrazolium (NT) affords certain advantages when compared with other tetrazolium compounds in terms of the speed of reduction, stability of the formazan, and toxicity. For these reasons, NT has been employed in the studies herein reported. Previously, Stein and Gerarde [8] reported no reduction of triphenyltetrazolium chloride (TTC) in chick heart fibroblasts, while Weiss and Fawcett [9] were unable to obtain reduction of blue tetrazolium (BT) in chick monocyte cultures with added succinate. Knoth [10] observed reduction of TTC in guinea pig spleen fibroblasts but not in chick heart fibroblasts. In a more recent report, Marcuse [11] described NT reduction in cultures of human neoplasms in the presence of succinate, but observed no reduction in fibroblastic outgrowths of the explant.

The present investigation was undertaken in order to determine the metabolic activity of fibroblasts by histochemical means. The enzymatic characteristics of chick embryo fibroblasts in tissue culture were studied with NT alone and in the presence of a variety of substrates and cofactors.

Materials and Methods

(1) *Preparation of fibroblast cultures.* Ten-day-old chick embryos were taken out of the egg, the eyes and livers removed, and the remainder washed in two changes of Hanks' solution**(pH 7.2 to 7.4). The embryos

*This paper, illustrated with lantern slides, was the second of five papers in a symposium on Recent Advances in the Biological Application of Tetrazolium Salts presented at a meeting of the Section on February 14, 1955.

This study was aided by grants from the Lasdon Foundation and the Loyal League Philanthropies.

**150 units of penicillin and 100 μg. of streptomycin were added to each cc. of Hanks' solution.

were then squeezed to a pulp through a 50 cc. syringe, using 2 cc. of Hank's solution per embryo. After standing for 30 minutes, the emulsions were centrifuged at 2,000 r.p.m. for 10 minutes. The cell sediment was treated with trypsin (0.4 per cent) at 37° C. for 15 minutes; the trypsin was removed by washing the sediment in Hanks' solution and centrifuging. This procedure was repeated three times. The cells were then placed in a mixture consisting of 3.5 cc. of Hanks' solution, 1 cc. of horse serum, and 0.5 cc. of embryo extract in a small petri dish, and adjusted to a final suspension of 5×10^6 cells per cc. The cultures were grown at 37° C. for three days in an oven gassed with 5 per cent CO_2 in oxygen, to maintain a pH below 8.

(2) *Study of NT reduction in the fibroblast cultures.* To these three-day-old cultures, 5 cc. of a 0.5 per cent isotonic phosphate-buffered NT solution was added alone and with the following substrates: glutamate, acetate, glycerine, succinate, lactate-DPN, mannose, glucose, and alpha-glycerophosphate-DPN. One cc. of a 3 per cent stock solution of substrate was added. With substrates whose oxidative metabolism required the presence of coenzyme 1 (DPN), 0.5 cc. of a 0.5 per cent solution of DPN was added to the medium. Several cultures in NT alone were also treated with DPN.

Cultures were then incubated at 37° C. for 24 hours and examined microscopically. Much shorter incubation periods were insufficient to produce maximal deposition of formazan.

Results

TABLE 1

ESTIMATED TETRAZOLIUM REDUCTION WITH VARIOUS SUBSTRATES

No reduction	*Moderate reduction*	*Marked reduction*
NT alone	Succinate	Mannose
DPN	Lactate-DPN	Glucose
Glutamate		Alpha-glycerophosphate-DPN
Acetate		
Glycerine		

As shown in TABLE 1, no endogenous reduction could be detected with NT alone, even in the presence of DPN; likewise, with the addition of glutamate, acetate, or glycerine, NT was not reduced. NT was reduced, however, in the presence of succinate, lactate-DPN, glucose, mannose, or alpha-glycerophosphate-DPN.

The NT-treated cultures contained many multinuclear cells with prominent, and often multiple, nucleoli. Mitoses were not observed. Fat phanerosis was evident. In all cultures where NT reduction took place, the formazan granules were first observed in the perinuclear region and later were found throughout the cytoplasm of the cell. In several instances,

particularly with glucose as a substrate, the fibroblasts were rounded and detached from one another; with glucose, a spicular arrangement of the formazan was occasionally observed. In a previous study with three dimensional explants of chick heart, the fibroblasts reduced NT in the absence of substrate. Here also, fat phanerosis was noted in the preparation; the fat assumed a pink hue, apparently extracting formazan from adjacent structures.[12]

In an effort to define the enzyme mechanisms which play a role in the reduction of NT, malonate and fluoride were employed as inhibitors in a limited number of trials. With final concentrations of sodium malonate of 0.5 per cent or higher, there was complete suppression of NT reduction in the presence of succinate, but no inhibition in the presence of glucose. Concentrations of malonate of the order of 0.02 per cent appeared to augment NT reduction in the presence of both succinate and glucose. Sodium fluoride, in concentrations of 0.05 per cent or higher, resulted in almost complete inhibition of NT reduction in the presence of glucose and mannose, but was without effect in the presence of the other substrates employed.

Discussion

Fibroblast cultures incubated with NT in the absence of specific substrates failed to demonstrate endogenous reductase activity. This failure indicates that the formazan deposition in the fibroblasts treated with a variety of substrates is probably a reflection of the activity and possible spatial location of the specific enzymes acting on the individual substrates. This assumption is supported by the observation that malonate, which is a specific competitive inhibitor of succinate dehydrogenase,[13] blocks NT reduction in the presence of succinate. Since tetrazolium reduction occurred in the presence of the hexose sugars, without added DPN, this reduction may be attributed to a general potentiation in activity of the glycolytic cycle, rather than to activation of specific hexose dehydrogenase activity. This view is supported by the fact that glucose dehydrogenase is known to be DPN-dependent and by the observation that fluoride, which blocks the glycolytic cycle,[13] selectively inhibited NT reduction only when glucose was used as a substrate. The similarity of glucose and mannose in producing NT reduction suggests a possible pathway in cellular metabolism which incorporates mannose into the general glycolytic system. In a previous investigation, one of us[14] demonstrated the presence of enyzmatic pathways for the metabolism of mannose in vascular tissue.

Although much attention has been focused on the histological demonstration of comparatively few enzyme systems, using the tetrazolium salts as indicators,[15,6] it can be seen that a wide variety of enzymatic reactions may be explored with these agents. Thus, in the fibroblasts herein investigated, high alpha-glycerophosphate-DPN dehydrogenase activity

was demonstrated. The absence of NT reduction by fibroblasts in the presence of acetate, glutamate, and glycerine indicates that the enzymatic apparatus governing the metabolism of these substances cannot be measured with the technique herein employed.

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SECTION OF BIOLOGY

THE EFFECT OF NEOTETRAZOLIUM ON VIRUS GROWTH*

By Helen Kodza and William Antopol

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Since tetrazolium compounds affect growth and other vital cell processes,[1,2,3,4] it seemed of interest to determine whether these compounds would influence virus multiplication in the living cell.

The first studies dealt with the *in vitro* growth of influenza virus A PR8, in the neotetrazolium (NT) pretreated chorioallantoic membranes of developing chick embryos. Later studies dealt with the growth of western equine encephalomyelitis virus (WEE) in chicken fibroblasts.

Material and methods. The balanced salt solutions (BSS), Simm's saline (SS), and Hanks' solution used contained 150 units of penicillin and 100 μg. of streptomycin per cc.

Phosphate buffer saline (PBS) was used as in the Dulbecco technique.[5]

Trypsin. 0.4 per cent Difco trypsin in Hanks' solution.

Viruses used. Influenza virus A PR8 in allantoic fluid received from the Public Health Research Institute of the City of New York, N. Y. This had a titer of 8.5 ID_{50} per cc. in embryonated eggs. WEE virus was received from Doctor R. Dulbecco in fluid from chick fibroblast culture. Its titer was 1.9×10^8 plaque-forming units on fibroblasts and 8.7 LD_{50} per cc. tested in chick embryos. Stocks were kept at $-20°$ C.

Tissue culture and titration methods. Chorioallantoic membranes from 10-day-old embryos were washed four times in PBS, cut into three or four pieces each, and pooled. Fragments, corresponding to the equivalent of two eggs, were suspended in 15 cc. of Simm's saline in a petri dish containing various concentrations of NT. After 18 hours, the membranes were washed four times in PBS and placed in a new dish with medium, and the virus was then added. After three days at 37° C. the tissue was washed four times in PBS to remove free virus particles and titrated for infectivity in eggs. The fluid medium was similarly treated. The membranes were ground with sand in a mortar and suspended in chilled broth to make a 10 per cent concentration. This was centrifuged at 2000 r.p.m. for five minutes, fivefold or tenfold dilutions were made in broth and 0.1 cc. was injected into the allantoic cavity of four nine-day-old embryos for each dilution. The eggs were incubated at 37° C. for 48 hours, chilled overnight, and the allantoic fluids were assayed for

*This paper, illustrated with lantern slides, was the third of five papers in a symposium on Recent Advances in the Biological Application of Tetrazolium Salts presented at a meeting of the Section on February 14, 1955.

This study was aided by grants from the Lasdon Foundation and the Loyal League Philanthropies.

agglutinins by the Hirst[6] method. The results were calculated according to the Reed and Muench[7] method to determine the 50 per cent infectivity end points. The culture fluids were titrated by the same method.

For *fibroblast cultures*, free cells were prepared by pressing 10-day-old embryos through a 50 cc. syringe into a flask with two cc. of Hank's solution per embryo. This culture remained at room temperature for 30 minutes and was centrifuged for 10 minutes at 2000 r.p.m. The supernatant fluid was again centrifuged for 10 minutes and was used as chick embryo extract (CEE). The slimy deposit from the first centrifugation was treated with trypsin in a water bath at 37° C. for 20 minutes. The trypsin was washed out by centrifuging and resuspending three times in Hanks' solution. The cells were counted and suspended in a medium of seven parts Hanks' solution, two parts horse serum, and one part chick embryo extract. Then 8×10^6 cells in 10 cc. of this medium were placed in 100 mm. petri dishes, and 5×10^6 cells in five cc. of medium were placed in 60 mm. petri dishes. The dishes were next placed in an incubator which was gassed for two to three minutes with a mixture of 5 per cent CO_2 in oxygen to prevent the pH from rising above 8. The incubator was not opened at any time during the three-day incubation period. The monolayer growth of fibroblasts was washed once with five cc. of PBS, the virus inoculum was added and absorbed at 37° C. for 30 minutes. The growth was then washed twice with PBS and covered with five cc. of medium, composed of four cc. of Hanks' solution and one cc. of CEE. The cultures were kept in the gassed incubator at 37° C. and at intervals 0.5 cc. samples were collected for plaque-method titration.[5]

For titration by the plaque technique, 0.3 cc. of the virus dilution was added to the fibroblasts grown in the 60 mm. petri dishes. This was adsorbed for 30 minutes at 37° C. and covered with 3.7 cc. of agar overlay. (For the overlay, 1.2 cc. of a 2.7 cc. solution of unwashed Difco agar was placed in tubes and autoclaved. The melted agar content of each tube was mixed with 2.5 cc. of a mixture consisting of 0.8 cc. Hanks' solution per tube, 0.5 cc. CEE, and 1.2 cc. of 1:10,000 neutral red solution in water. The mixture was kept at 44° C. for 10 minutes prior to mixing.) The dishes were then placed in the gassed incubator at 37° C. The plaques were counted after three days.

Neotetrazolium (NT). A 6.7×10^{-3} M.NT solution in saline was autoclaved and used as a stock solution.

Nuclei count. The fibroblasts were washed once, covered with four cc. of 0.1 M. citric acid and one cc. of 0.1 per cent crystal violet, and kept at 37° C. for two hours. The cells were scraped off, mixed by vigorous pipetting, and then counted in a hemocytometer.

Results

Individual membrane cultures were treated with NT in concentrations

varying from 1:40,000 to 1:2,560,000, and influenza virus was inoculated. FIGURE 1 indicates that, in membranes treated with 1:40,000 NT, far less virus was found while, with concentrations of 1:640,000 and 1:128,000, the virus titer of the extracted membranes was one logarithmic unit higher than in the control.

FIGURE 2 represents 30 cultures. Fifteen were used as controls and the others contained 1:1,280,000 NT. It is apparent that the amount of virus in the NT-treated membranes was higher than in the controls (0.8 to 1.5 logarithmic units), and that NT-treated membranes were infective up to 20 days. The virus titer was also higher in the fluid phase.

FIGURE 3 illustrates an experiment, in which nine control membranes and nine NT-treated membranes (see above) were prepared in an attempt to infect the majority of the cells in one step, with the possible release of the virus of one cycle. $2 \times 10^7 ID_{50}$ was inoculated in each culture. The virus extracted from the NT-treated membranes and the virus released into the fluid were both higher than in the controls. Furthermore, six days after the infection the NT-treated membranes contained four logs more of the virus than the controls.

In order to determine the length of time that NT-treated membranes could be kept and still retain their ability to be infected by virus, NT-treated membranes were kept at 37° C. for 10, 12 and 15 days and

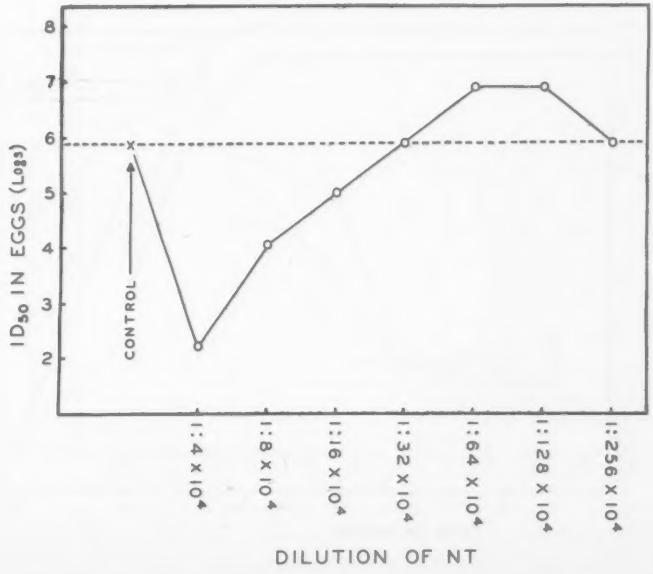

FIGURE 1. Infectivity titer of membranes treated with varying concentrations of NT.

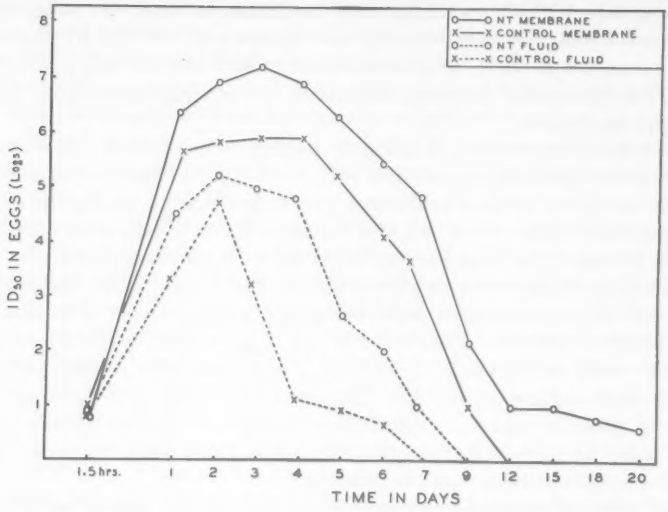

FIGURE 2. Growth curves of NT pretreated membranes and controls *in vitro*. Virus inoculum 10^3 ID_{50} NT concentration 1:1,280,000.

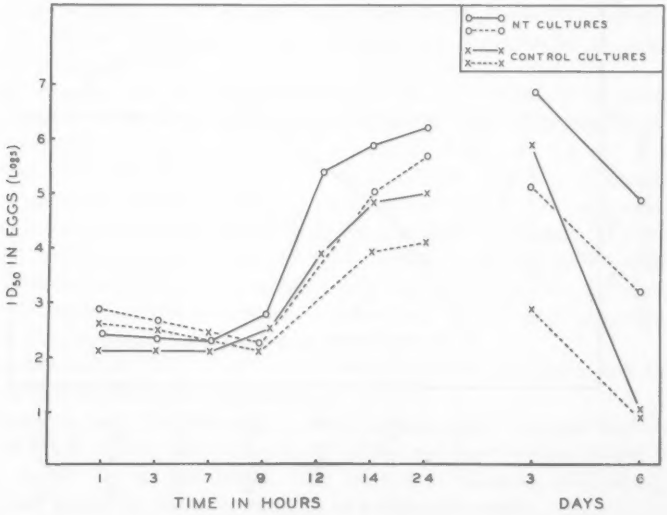

FIGURE 3. Results of "one step" growth experiment with NT treated membranes. Virus inoculum 2×10^7 ID_{50} NT concentration 1:1,280,000.

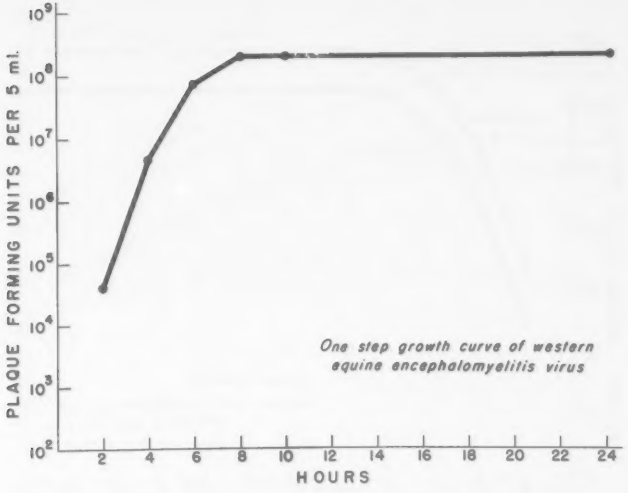

FIGURE 4

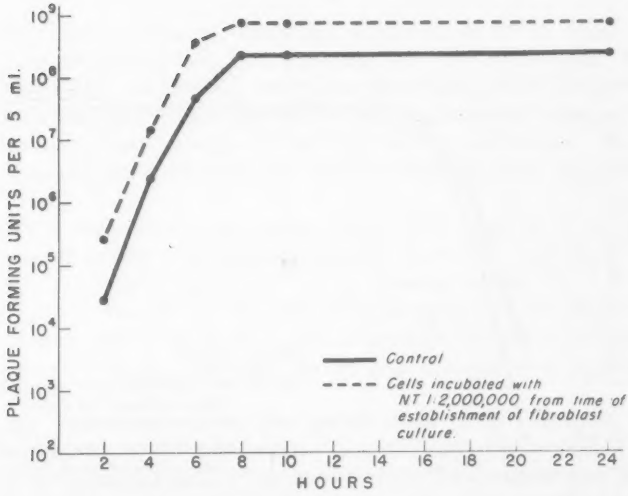

FIGURE 5. One step growth curves of WEE virus.

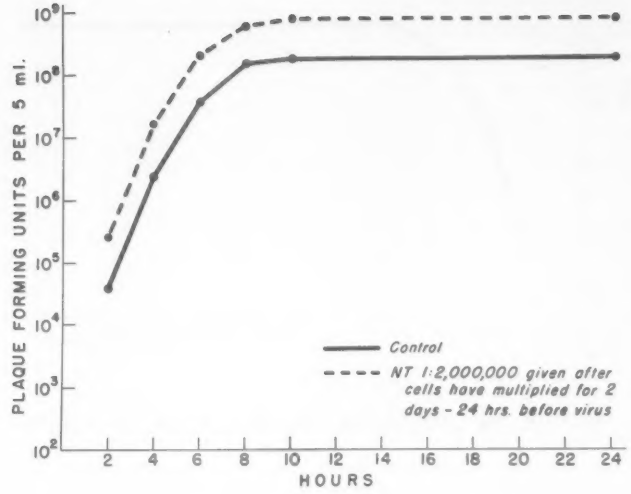

FIGURE 6. One step growth curves of WEE virus.

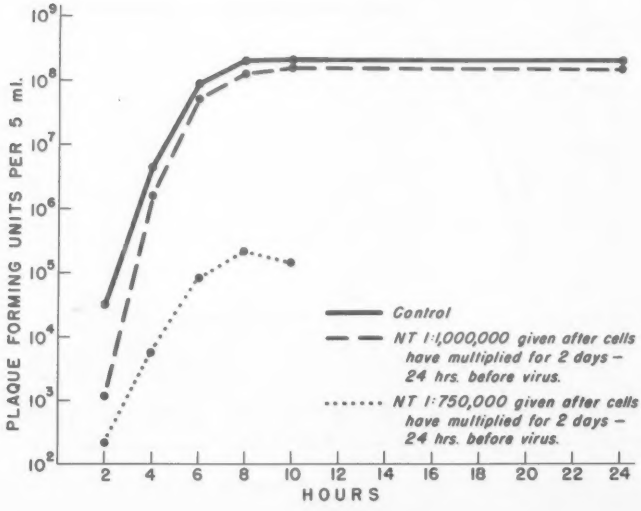
PLAQUE FORMING UNITS PER 5 ml.
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
2 4 6 8 10 12 14 16 18 20 22 24
HOURS
Control
NT 1:1,000,000 given after cells have multiplied for 2 days – 24 hrs. before virus.
NT 1:750,000 given after cells have multiplied for 2 days – 24 hrs. before virus.

FIGURE 7

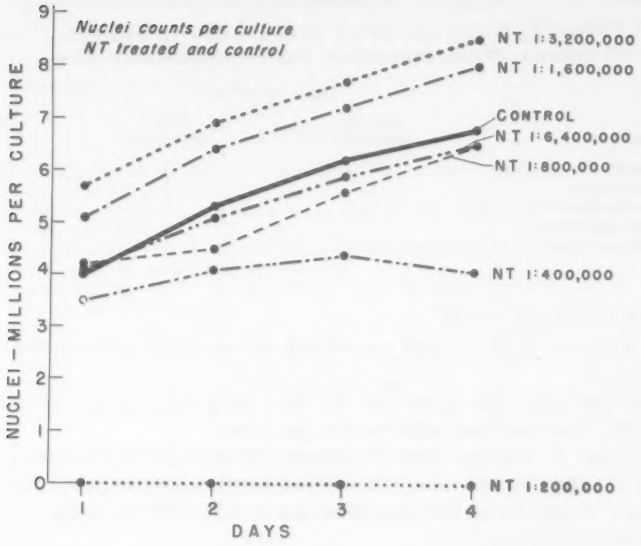

FIGURE 8

then were infected with the virus and incubated at 37° C. TABLE 1 shows that membranes kept in 1:1,280,000 NT for 12 days and then infected revealed 2.10 logs ID_{50} infectious virus, while control membranes contained less than 2 logs when infected after 10 days' preservation.

Dulbecco's plaque technique [5] was used as a more accurate quantitative method, with WEE virus and chicken fibroblasts as susceptible cells.

FIGURE 4 represents a one-step growth of WEE virus on fibroblasts. The three-day growth was absorbed with 0.5 cc. of 10^{-1} virus at 37° C. for 30 minutes (containing 1×10^7 plaque-forming units). Since three-day-old cultures contain 1×10^7 cells, almost every cell is infected by one virus unit. After a two-hour lag phase there is a phase of increasing release which lasts for four hours and is then followed by a stationary phase.

FIGURE 5 represents a one-step growth curve on two cultures. The virus titer in the NT-treated cells is higher than in the control. Each point is the average value of four agar dishes. This process has been repeated four times, so that each point on the graph represents the mean of 16 trials. The values, subjected to statistical analysis, have indicated that the probability of this conclusion being a chance finding is 25:1000. Nuclei counts of one NT culture and one control, prepared at the same time, gave a count for the NT culture of 3×10^7 and for the

TABLE 1

MEMBRANES KEPT AT 37° C. FOR VARYING PERIODS BEFORE VIRUS INFECTION. VIRUS INOCULUM 10^3 ID_{50}

NT 1:1,280,000	*Days at 37° C. Before infection*	*After infection*	*ID_{50} in eggs*
NT membrane	10	3	3.40
NT membrane	12	3	2.10
NT membrane	15	3	< 2
Control membrane in buffer saline	10	3	< 2

control a count of 1×10^7.

In FIGURE 6 the plotted curves are almost identical with those in FIGURE 5.

After the cells had grown for two days on a sheet they were treated with NT. The virus was added on the third day.

FIGURE 7 indicates that fibroblasts treated with NT concentrations under 1:1,000,000 and then infected had infectious titers lower than the controls. Furthermore, NT concentrations of 1:750,000 markedly inhibited virus multiplication.

NT, mixed with the virus and then added to the cells, has a lethal effect on the free virus particles even in concentrations found to be stimulatory to the cells in which the virus grows.

To study the effect of NT on the cell growth, a number of cultures were prepared with 1×10^6 cells and varying concentrations of NT. These cultures were not gassed. After 1, 2, 3, and 4 days at 37° C., one culture of each series was sacrificed and the nuclei counted. The nuclei count was taken as the number of grown cells. FIGURE 8 indicates that, with 1:200,000 NT, the fibroblast cells do not stick to the bottom of the dish and therefore cannot be counted. In concentrations of 1:400,000 of NT, the nuclei were far below the control. In concentrations of 1:1,600,000 and 1:3,200,000 NT, the nuclei counts were higher than the control. This result does not necessarily indicate cell division only, since NT has a tendency to produce many multinucleated cells in tissue culture.[8]

Discussion and Summary

The present study indicates that NT exerts a lethal effect on influenza and WEE virus. However, if low concentrations of NT within a narrow range act on the host cells 8 to 24 hours before the addition of virus, more virus is produced. This effect has been found in two different systems: with influenza infected chorioallantoic membranes and with WEE virus-infected fibroblasts. In a concentration of 1:640,000 for the former and 1:1,000,000 for the latter, NT has a stimulatory effect. With

similar concentrations, the nuclei counts are higher in the fibroblast cultures. The increase in the virus production may be dependent primarily on the greater number of host cells or on an increase in the virus yield per cell.

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SECTION OF BIOLOGY

QUANTITATIVE AND HISTOCHEMICAL USAGE OF TRIPHENYLTETRAZOLIUM IN PATHOLOGY

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The reduction of the water-soluble, colorless tetrazolium salts by cellular enzymatic activity yields a pigmented water-insoluble formazan which is deposited in close proximity to the site of such activity. Since such formazan deposition may be observed directly and measured quantitatively, it is evident that the same agent may serve to measure the intensity as well as to demonstrate the site of such enzymatic reductions within a complex tissue.[1, 2, 3] In addition, it has been observed that the type of formazan deposition may vary within the same cell type under different physiological conditions.[4,5] It is thus possible to demonstrate an additional parameter for characterizing the tissues or cells under investigation.

The data to be presented at this time are based exclusively on observations on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) by tissue slices. The basic incubation medium employed consisted of three ml. of 1 per cent aqueous solution of TTC plus one ml. of 0.9 per cent NaCl, buffered to pH 7.2. This medium is used for the evaluation of the endogenous dehydrogenase activity. In studies of the effects of various substrates and inhibitors, the saline is replaced by the substrate or inhibitor. Histochemical and quantitative comparisons are then made between the endogenous activity and the activity observed in the presence of the experimental agents studied.[6, 7, 8, 9]

While TTC has been used to investigate a wide variety of pathological conditions, the data to be presented were chosen to illustrate the variety of problems to which TTC is readily applicable. As indicated above, TTC may be used both as a histochemical and as a quantitative tool. Furthermore, the histochemical usage may provide information as to (1) the relative intensity and localization of metabolic activity in the components of a complex organ or tissue, and, (2) evidence of altered metabolism visualized by differences in the type of formazan deposition. Illustrations of these various usages of TTC are presented in the following examples.

(1) *Histochemical*

(a) *Intensity and localization of metabolic activity in a complex organ or tissue.* A simple and direct demonstration of the value of the TTC

*This paper was the fourth of five papers in a symposium on Recent Advances in the Biological Application of Tetrazolium Salts presented at a meeting of the Section on February 14, 1955.

techniques is found in studies of the metabolic activity of slices of mouse mammary carcinoma. Incubation of slices of such tissue in TTC reveals that the reduction of the dye takes place almost exclusively in the tumor stroma, particularly the capillaries. Without such histochemical observations one would be inclined to falsely ascribe the major respiratory activity of the tissue to the tumor cells. [10]

The TTC techniques are also applicable to the problems of functional alterations in complex glands, *viz.*, the adrenal gland. [11,12] The endogenous reduction of TTC by adrenal gland slices reveals a prominent red coloration of the lipid granules in all the zones of the cortex and minimal to no staining in the medullary region. Variations in this basic staining pattern may be observed under different experimental conditions. Thus the *in vivo* injection of DOCA into intact rats results in a loss of TTC reduction by the *zona glomerulosa* which precedes the eventual loss of lipid and atrophy of this zone. On the other hand, cortisone injections produce a loss of staining of the inner zones which precedes the eventual atrophic structural changes seen in these zones.

In a study of the *in vitro* metabolism of adrenal slices from control and tumor-bearing mice, subtle differences are also demonstrable with the TTC technique. [13] In these experiments, adrenal slices from control males, control females, and tumor-bearing CFW female mice were incubated in TTC with and without added steroid hormones. This technique proved capable of demonstrating a differential response to the different steroids in accord with the physiological state of the host. [14] Thus the male adrenals were characterized by inhibition by DOCA, estrogen, and compound A, minimal changes by testosterone, and stimulation by cortisone and compound F. The female controls responded in a similar fashion to the males except that no inhibition by estrogen was observed. In contrast, adrenal slices from females bearing spontaneous breast carcinomas were inhibited by cortisone, compound F, testosterone, estrogen and compound A, while DOCA produced only minimal change or actual stimulation in some cases. Furthermore, the *in vitro* effects of the steroid hormones were most prominent in the *zona glomerulosa* and minimal in the inner zones of the cortex. It is important to emphasize that no significant differences were detectable in the adrenals of the different groups as judged by conventional hematoxalin- and eosin-stained preparations, or by the use of lipid-stained preparations. It is thus evident that TTC techniques are capable of visualizing subtle functional changes occurring without structural alteration.

(b) *Variations in type of formazan deposition.* In previous reports we called attention to the change in the type of formazan crystals in the proximal convoluted tubules of kidneys from hypertensive humans and animals as compared to the formazan deposition in control kidneys. At this time I should like to illustrate a similar phenomenon as observed in

the human lymph nodes and spleens under different conditions.[15] In the normal lymph node the formazan deposition is largely confined to the reticular cells of the stroma with only minimal staining being observed in the lymphocytes. In node slices from lymphosarcoma, there is a well-defined punctate staining in the cytoplasm of the tumor cells. Similarly, in chronic lymphatic leukemia there is also a granular formazan deposition in the leukemic cells which takes the form of coarse rodlets. In Hodgkin's disease the appearance is similar to that of the control nodes, except that the staining is more prominent in the reticulum cells and stroma wherein the formazan is deposited in elongated crystals. A similar but even more accentuated elongated type of crystal staining is observed in the granulomas of sarcoidosis and tuberculosis. In the normal human spleen slices, the TTC reduction is similar to that of the control lymph nodes, *viz.*, minimal TTC reduction by the lymphocytes of the pulp or follicles. In spleen slices from cases of hypersplenism, however, a well-defined granular formazan deposition is found in the lymphocytes.[16] These data indicate the ability of the tetrazolium techniques to demonstrate differences in the cellular components of a complex structure by virtue of the type of deposition, as well as by its location and intensity.

(2) *Quantitative*

It is self-evident that quantitative measurements of any type may be applied to a wide diversity of problems in pathology.[3,17] It would be pointless, therefore, to attempt any enumeration of the various quantitative measurements that have been made by us in our laboratory, as well as elsewhere by others. I should like to illustrate, however, the usage of the tetrazolium techniques in order to present information of a type not provided by the usual and conventional pathological procedures. In essense, the problem was concerned with an attempt to predict the clinical benefits of cancer chemotherapy and radiation therapy of individual cancer cases.[8,9] To this end, we studied the *in vitro* effects of urethane, TEM, and TEM plus urethane on the TTC reduction by tumor tissue slices. Measurements made of the micrograms of TTC, reduced per mg. tissue in the presence and absence of the experimental agents, provided an evaluation of the per cent change. It was found that radiosensitive cancer tissues were characterized by an appreciable *in vitro* urethane inhibition of their dehydrogenase activity. Conversely, radioresistent cases showed only minimal urethane inhibition or actual stimulation. Similar correlations were also found between the *in vitro* and clinical effects of TEM and TEM plus urethane. Such findings were not predictable on the basis of the microscopic structure of the tumors. It is thus evident that vital metabolic measurements may provide information of significance to the study of pathological alterations in tissues.

The various data presented illustrate the applicability of the tetrazolium salts to the investigation of diverse problems in pathology. It

should be mentioned, however, that there is need for a thoroughgoing investigation of the advantages and limitations of the different tetrazolium salts as applied to the measurement of functional activity of different tissues under varying states of activity. It is hoped that the more widespread usage of these techniques will help to define the optimum procedures to be employed.

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SECTION OF BIOLOGY

TETRAZOLIUM SALTS AS INDICATORS OF CHANGES IN TISSUE METABOLISM DURING FUNCTIONAL ACTIVITY*

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Various tetrazolium salts have been widely used in recent years as indicators of general reduction and specific dehydrogenase activity in biological material. Although the particular metabolic implications of this reaction remain for the most part speculative, it is hoped that our increasing knowledge of the experimental factors concerned with the reduction of these agents by cellular systems will make possible a more finite extrapolation of the data. Fresh tissue slices [1,2,3] as well as fresh, frozen section techniques have been employed.[4,5,6,7] Each method has its values and also its drawbacks. Before undertaking a broad application of these techniques, however, it seemed essential to establish a suitable medium which would also assure reproducible results. The most satisfactory incubation solution was an isosmotic solution made up of a modified Krebs' solution[8] with an appropriate tetrazolium salt (TABLE 1). In the present studies, 2,3,5 triphenyltetrazolium chloride (TTC) and neotetrazolium (NT) were used.

Endogenous studies have been carried out on mesenteries (which are convenient locations for the observation of peripheral blood vessels) and also on organ slices. In the case of the mesenteric structures, it has been found that the present medium (TABLE 1) makes it possible to obtain reproducible results in the reduction of NT by vascular elements

TABLE 1

MODIFIED KREBS' MEDIUM WITH TETRAZOLIUM SALT
(pH 7.3 - 7.6)

Salt	*Per cent*		*Parts*
NaCl	1.70		100
KCl	1.15		8
KH_2PO_4	2.11		2
$CaCl_2$	1.22		6
$MgSO_4.7H_2O$	3.82		2
$NaHCO_3$	1.30		6
$Na_2HPO_4.2H_2O$	1.75	100 pts	
		:25 pts	6
$NaH_2PO_4.H_2O$	1.38		
Tetrazolium	1.00		100

*This paper, illustrated with standard and Kodachrone slides, was the last of a series of five papers presented at the meeting of the Section, February 14, 1955.

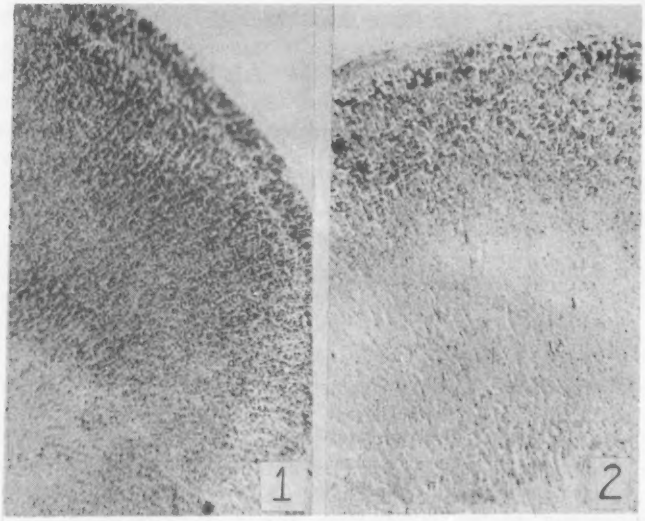
This investigation was supported in part by the A. Krasne Fund of the Beth Israel Hospital, New York, N. Y.

of the spread. TTC was found unsatisfactory for this purpose. Some earlier work, which did not make use of this modified Krebs' solution, required the addition of substrate to the basic NT-salt solution in order to obtain consistent reduction of the tetrazolium by blood vessels.[9] Together with adjacent tissue, mesenteries (mesovarium, mesorchium, mesoappendix, intestinal mesentery) were carefully removed from the animal (CF Wistar rats, CF mice), placed in ice cold NT-Krebs, and incubated either anaerobically for two hours or aerobically for two and one-half hours at 37° to 38° C. without agitation. The reaction was stopped at the appointed time by adding 10 per cent neutral formalin to the tissue. The spreads were observed with a dissecting microscope and were later mounted in glycerine and examined under higher magnifications. Peripheral nerves, blood vessels, lymphatics, and mesothelial cells showed an active reduction of NT. Purple formazan was deposited in the endothelium of capillaries, venules, arterioles, arteries, and lymphatics, as well as in the muscular layers of these vessels. Connective tissue fibers remained free of formazan. Preliminary work has indicated that abnormal changes in vessels, due to such agents as X-radiation or experimentally induced inflammation, may be reflected in the extent of formazan deposition.

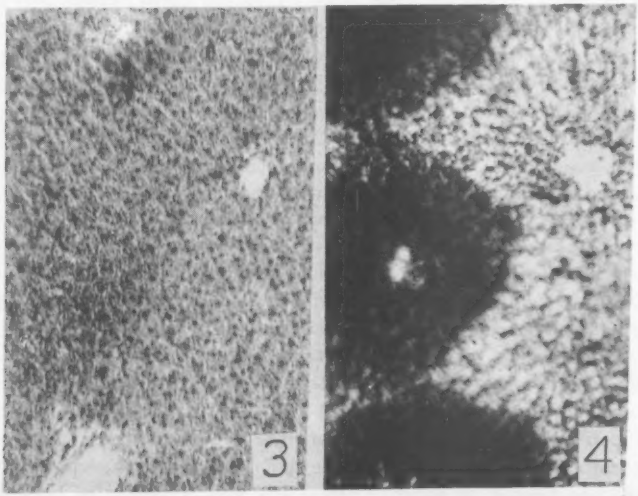
In endogenous studies of parenchymatous tissues, slices one mm. thick were prepared (as for Warburg work), placed in ice-cold tetrazolium-Krebs, and incubated anaerobically or aerobically from one to two hours at 37° to 38° C. Tissues were fixed in 10 per cent neutral formalin, sectioned at 15 to 20 μ with a freezing microtome, mounted in glycerine, and examined. Adrenal slices were poorly penetrated by NT whereas TTC penetrated well and showed uniform reduction throughout the cortex of control glands.[10] The formazan was deposited intracellularly, particularly in the lipid droplets (FIGURE 1). Under conditions of stress, there was a change in the pattern and extent of tetrazolium reduction and the type of deposition varied with the particular stress situation.

Rats kept on 1 per cent sodium chloride as drinking water for one month (33 days) were sacrificed, and their adrenals prepared for *in vitro* incubation studies as in control experiments. Microscopic examination showed a marked decrease in reduced intracellular TTC, particularly in the *zona glomerulosa*. Reducing activity was still evident in parts of the reticularis and fasciculata as indicated by intracellular formazan coloration. In addition, there was a heavy precipitate of extracellular cyrstals in all three zones of the cortex (FIGURE 2).

The effects on the adrenal of another type of stress (cold) were studied in rats kept at 5° C. for three and one-half hours. Adrenals were removed and treated as above. Cold stress was accompanied by a diminution of formazan in the reticularis and inner fasciculata, whereas the outer fasciculata, as well as much of the glomerulosa, continued to reduce TTC. In addition, there was a preponderance of extracellular



FIGURES 1 and 2. Sections of adrenals incubated endogenously in TTC-Krebs' solution under aerobic conditions. (1) Control adrenal showing intracellular formazan deposits uniformly distributed throughout the gland. ×100. (2) Adrenal of saline-treated animal showing a marked decrease in intracellular formazan especially in glomerulosa. × 100. Extracellular deposits are prominent.



FIGURES 3 and 4. Fresh frozen sections of liver incubated in NT-Krebs (with α-glycerophosphate as a substrate), under anaerobic conditions. (3) Control liver showing uniform deposition of formazan. × 60. (4) Liver of X-radiated animal showing the absence of formazan in damaged cells around central veins. × 100.

formazan in the fasciculata and glomerulosa.

There was also evidence of change in the pattern of formazan deposition in adrenals of rats which were sacrificed after X radiation. In the example to be cited, the rat was sacrificed two weeks after exposure to 750 r. (radiation had been delivered by a Machlett tube, 43 r. per minute, 200 kv., 20 ma., 49 cm. to center of the body, with added filtration of 1 cm. aluminium and 0.5 cm. copper). The lipid content in the gland was depleted, as was the intracellular formazan deposition. Extracellular deposits were extensive in the *zona glomerulosa* and outer fasciculata.

Endogenous studies of liver slices were not satisfactory because of the negligible penetration of both TTC and NT. Formazan was deposited only on the edge of the slice and just adjacent to the central veins. It was found that penetration was somewhat improved if slices were soaked 18 to 24 hours in the medium kept at O° C. before incubation (unpublished data). On the other hand, measurement of exogenous activity was much more susceptible to study. Fresh, frozen tissues were sectioned at 30μ and incubated at 37° to 30° for two hours anaerobically in the NT-Krebs medium, to which has been added specific substrates (10 parts medium to 3 parts substrate). The substrate dependent condition cited here utilized disodium α-glycerophosphate in a 0.1 M. solution. After incubation, sections were fixed in 10 per cent neutral formalin, mounted in glycerine, and examined.

The liver of control animals showed an over-all activity with uniform deposits of formazan in all cells (FIGURE 3). On the other hand, the liver sections of an animal sacrificed two weeks after a 750 r. dose of X radiation showed a complete absence of NT reduction in the damaged cells around central veins (FIGURE 4). In the case of saline-treated animals, liver sections did not seem to be different from controls.

It appears that tetrazolium salts are useful indicators for the study of changes in tissue metabolism during changes in functional state. It is necessary to select carefully the salt to be employed, as well as the method of handling the tissue, in order to provide pertinent information under different experimental conditions.

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SECTION OF MATHEMATICS AND ENGINEERING

PROBLEMS OF ANALYSIS OF STRESSES IMPOSED BY FOUNDATION LOADS IN SOILS SUPPORTING STRUCTURES*

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Foundation problems are as old as the building art itself. The development of foundation and earthwork engineering may be divided into three stages, starting from antiquity and continuing through the present. In the first stage, there was the development of practical methods of getting down to satisfactory bearing soils and of constructing foundations, such as the cofferdam, open caisson, and pneumatic-caisson methods in the 17th and 18th centuries. The second stage brought the development of the theory of lateral earth pressures exerted by back fills against retaining walls by Coulomb (1774) and the theory of stresses and displacements imposed in soils beneath foundation loads by Boussinesq (1885). These theories are based on ideal soils and ideal conditions. In the third stage, during the 19th century, progress was made in the knowledge of the physical nature and behavior of soils. The science of soil mechanics, developing out of these earlier works, treats soils as engineering materials having physical properties and responses which can be determined experimentally. The advance in soil mechanics was greatly accelerated when Doctor Karl Terzaghi, in 1925, demonstrated and emphasized that theoretical investigations and experimental determinations of the responses and behavior of soils under stress had important and direct applications to the analysis and solution of practical problems in foundation and earthwork engineering. Credit is due him for his pioneer work in formulating concepts and principles of soil mechanics on a sound physical basis.

Notable progress has been made in this field since the 1930's. In major engineering works, where difficult foundation and stability problems have been encountered, scientific soil mechanics have developed and provided the basic information for eminently satisfactory and economical solutions. There is a disturbing aspect in the present situation, however, which has come about principally from the pressure exerted by engineering practice—pressure for immediate simple working sets of rules and formulas for practical applications, which anyone can use. Thus, in this most important field of practical applications, particularly during the pressure of the war years, emphasis was sidetracked from the main purposes and objectives of basic scientific developments and of the use of scientific methods in engineering practice to rule-of-thumb and handbook expedi-

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on February 18, 1955.

encies. Possibly the greatest barriers to scientific and real progress in the immediate future may be the habits of empiricism and broad generalizations, the attitudes and conceptions and precedents that have been and are now being set in practice.

There is, however, a growing awareness among soil engineers of the necessity for upgrading the practice and professional status of soil and foundation engineering. This age, having fully demonstrated the value and importance of basic scientific developments, scientific caution, "common sense," and creative engineering imagination, should reject the idea of simple answers to admittedly complex and difficult questions and problems. In engineering, "adequacy" should not be permitted to become a fixed idea, but rather should be constantly and consistently revised upward to keep fully apace with increasing knowledge and experience. It should be realized that attitudes and conceptions predetermine the character and quality of judgments and practices and, hence the real adequacy and reliability of the results of soil investigations and the recommendations for engineering works. There is an essential need for improvement of attitudes and conceptions: first, with regard to soil investigations and soil testing and second, with regard to the evaluation of the stress conditions and other conditions that control in each situation.

The Problem of Soil Testing

At present, too little is really known about the character and properties of soils and about their responses to environmental conditions and to new conditions imposed by the construction of structures. The influences of these conditions are either incompletely evaluated or are generally ignored. Soil engineers should now consistently begin to search for, and to develop, more basic conceptions and principles of soil behavior in order to remove these large ignorance factors from soil engineering practices.

The major problems now are these: first, recognizing, comprehending, and explaining fully the real soil phenomena; second, developing and using more scientific, fundamental, and adequate approaches and sensitive, refined techniques in soil investigations, both experimental and theoretical, that are capable of a growth and improvement fully in keeping with scientific advances; and, third, consistently achieving the greatest possible agreement between predictions of soil behavior and the actual observed phenomena during and after the construction of structures.

It should be clearly recognized that one is actually dealing with a very unconventional and, in many respects, a very unusual kind of material in work with soils. Hence, in contrast to the essential uniformity of the common structural materials, the predictability of their behavior within the range of common working stresses and the marked constancy of their properties for all common conditions of usages unaffected by external conditions, soils should not be expected to follow such simple con-

ceptions and patterns of behavior. These facts are summarized in two basic concepts:[1]

(1) "The character and responses of soils in a particular situation not only are predetermined by, and are a part of, the environmental conditions prevailing in that situation, but they are always markedly conditioned and modified subsequently in direct response to the new controlling conditions imposed by structures.

(2) "In each situation, as a particularized case, the character and potential behavior of the soils obtained from soil tests must be considered directly in relation to the specific conditions that now control, whether different conditions are inherent in different locations in the natural situation, or whether the imposed conditions are different."

Every soil, in its character and responses, and every situation, in the relative dominance and degree of control of its prevailing conditions, should be expected to be different in significant and even unusual respects. These differences should be considered self-evident truths, which have broad implications in soil and foundation engineering. Paralleling these two concepts regarding the conditioning of the character and behavior of soils by the environmental and imposed conditions, there is a third basic concept regarding the dominating influences of conditioning in soil testing:

(3) "During the measurement of properties and responses of soils in soil tests, the observed properties and responses themselves are conditioned, modified, and changed by the very procedures and test conditions used to an important but unknown degree, unknown because there are no absolute response bases for references. Thus, the measured properties and responses are not necessarily those having any significant and direct relations to the actual properties and responses of the soils under the actual controlling conditions that prevail or are imposed in the natural situation. Therefore potential behavior only can be learned from soil tests."

The applications of these three concepts are directed specifically toward removing ignorance factors, elements of uncertainty, and guessing from soil and foundation engineering. If these barriers are permitted to exist, they always penalize the favorable aspects in a situation so that full advantage can not be taken of them, and they obscure the unfavorable or adverse aspects so that they can not properly be evaluated and taken into account. As a basic conception and attitude, every soil test and analysis in soil investigations for engineering work should be considered as design in the true sense of the word, because such tests and analyses are really works of discovery to disclose, fully to evaluate, and properly to take into account, as a particularized care, the specific conditions that control in each situation. It should be realized that soil is a material in a complex situation, and that it does not readily permit, if at all, of translating responses obtained under one set of relatively arbitrary and artificial conditions in a standard test into reliable and adequate predic-

tions of responses under a quite different but real set of actual conditions in the natural situation. A more basic and reliable soil testing approach is required in accordance with the following principles of "controlled test methods":[1, 2]

(1) To appraise and to evaluate more completely the real nature and degree of control of the environmental conditions inherent in the natural situation and in the subsequent changed and new conditions imposed in sequence by the construction of structures, and the structural influences on soil character and soil responses.

(2) To translate this information into representative and significant "test conditions," which essentially reproduce or restore the natural environmental conditions that control in the field and to establish thereafter, in sequence, the stress and other conditions to be imposed by the construction of structures.

(3) To apply these test conditions by proper techniques, control, and adjustments during the conduct of soil tests, so that these measured responses of the soils which are in significant agreement with the phenomena observed during construction would have direct, reliable, and valid applications in predicting field performances under actual conditions.

There are certain objectives that should serve to give direction and incentive to improving the adequacy and reliability of soil testing and should remove the ignorance factors from soil investigations: first, the favorable aspects in a situation should be recognized and full advantage should be taken of them; second, the possibilities of improving conditions with regard to any unfavorable or adverse aspects should be fully explored and planned for in advance but, in any case, they should be taken fully into account in the planning and design of foundations and earth works; and third, there should be a consistent attempt to achieve the greatest possible agreement between the predicted soil behavior and the actual observed phenomena. These objectives can be accomplished by *fitting* design and construction methods more closely to actual existing conditions.

Evaluation of Stress Conditions Imposed in Soils

Investigations of the magnitude and distribution of stresses imposed in the supporting soils of structures by the foundation loads of structures are essential and important aspects of foundation and stability studies. The principal problems involved in the design of satisfactory and economical foundations for structures are those of limiting settlements and lateral movements to nonobjectionable values, and of insuring stability conditions against failure.

The theory of elasticity has provided working hypotheses and techniques for the analysis of stresses and displacements in soils, which are of great value and practical importance. Although the theories are based upon conditions of ideal materials and idealized boundary conditions, they have been found to be useful in the range of imperfectly elastic and and somewhat nonhomogeneous materials, such as soils, and they have demonstrated their practical value in estimating magnitude and distribution of stresses imposed by foundation loads in soils. But it would be misleading to suppose that all a problem demands is the use of formulas and tables in order to make a stress analysis. The value of thorough study and analysis is to learn more completely the real nature of a situation and to estimate the "probable."

Prior to about 1940, the Boussinesq problem[3] was the only working hypothesis available for stress analysis in soil. Since that time, theoretical advances of great importance have been made which are in closer agreement with actual soil conditions encountered in foundation practice. These advances are covered in a series of basic *stress problems*. It is essential to establish the realm of validity for each problem in its practical application to a particular situation regarding the specific boundary conditions inherent in the development of the problem in relation to actual boundary conditions that control in a particular situation. Each new advance in soil mechanics require a re-evaluation of present practices and methods of investigation, and requires the use of improved and more refined methods in all related aspects, in order to take full advantage of the advance. Such refinements should then be looked upon no longer as refinements, but as essential aspects of the advance. In considering these basic stress problems uniformly, distributed loadings, which have been obtained by the application of the principle of superposition of stresses, have been used, being more realistic and in greater agreement with foundation loading conditions as practiced.

Realm of Validity of Stress Problems Regarding the Behavior of Soils Under Stress

Some important aspects of stress problems will be considered here briefly in order that the investigator may be cognizant of the nature of the problems involved, the validity of his analysis, and the order of real accuracy of the results of his investigations. In the first place, foundations are seldom founded on the surface of the ground, but practically always at some depth. Hence, none of these basic stress pictures are strictly valid for this case. The usual practice of computing stresses below the foundation level and of ignoring the influences of the surrounding unexcavated soil introduces ignorance factors which can not as yet be evaluated. Suffice it to say here that the stresses estimated at the edges of the foundation adjacent to the unexcavated bank of soil are estimated too small by this practice. This knowledge, of itself, is of value, because some weight can be given to this fact.

In the second place, unlike the analysis of all stresses in structural problems, soils do not exist in the unstressed and unstrained state, but always in a state of "prestress," (as in prestressed concrete), which is a most important and favorable fact. With the refinements now available in the improved stress problems of cases 2 to 5, it now becomes necessary adequately and reliably to take into account this significant and dominant state of prestress in soils in applying stress analyses to problems of settlements of structures. The state of prestress is due either to the weight of present overburden of soil above any elevation, to other stress conditions imposed in a soil deposit during its past geological history, or to excavation for construction. The higher the state of prestress, the more favorable is the situation with regard to settlements.

The only possible way of taking this important state of prestress in real natural soil deposits is through the consolidation of stress-strain relations or the triaxial stress-strain relations themselves by the direct application of the three principles of controlled-test methods. The state of prestress is determined, during the test itself, by the responses of the soil to stressing, and is restored upon the specimen. Thereafter, the stress conditions to be imposed in sequence by construction of the structure are imposed on the specimen. Thus, reliable and valid stress-strain relations are obtained which reflect and take into account the state of prestress as the starting point of the stressing and straining of the soil under the imposed loading conditions in sequence, for example, the unloading of a deposit by excavation to foundation levels, followed in sequence by the reloading of the deposit to the final foundation loads, with the settlements now favorably reduced by the unloading.

Thus these favorable aspects in a situation can be disclosed and evaluated, and full advantage can be taken of them. Frequently conditions can be further improved by developing the design of foundations, and construction methods, and sequences more closely to actual existing conditions.

Case 1. The Boussinesq problem[3] gives the stress conditions in the supporting soils of a homogeneous deposit beneath a uniformly distributed foundation load applied at the surface of the ground, which is free of normal and shearing stresses outside the limits of the loaded area. This case is valid for a deposit which is reasonably homogeneous (not visibly layered) in character and for properties within a critical depth, as illustrated in FIGURES 1 and 2. For individual spread footings, this depth is equal to about four times the half-width (least) of the footing. For an entire foundation, the critical depth is defined, as a first approximation, as the depth at which the vertical stresses σ_z imposed on the soil decrease to a value equal to 20 per cent of the overburden stress at that depth for more compressible clay soils. The stresses are greatest directly beneath the foundation. A favorable aspect is the marked decrease in stresses with depth. It is to be especially noted, however, that for larger founda-

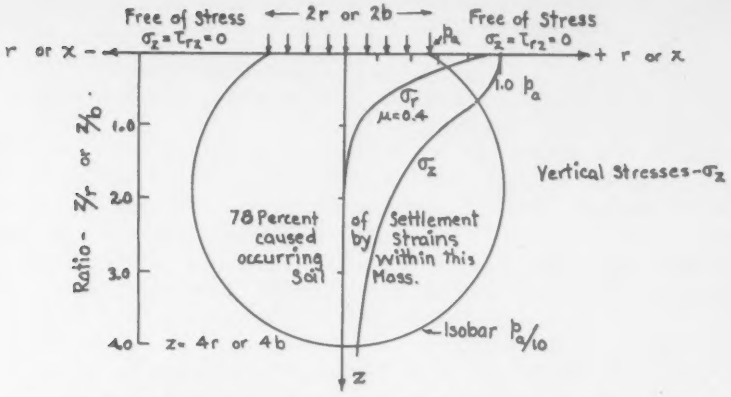

FIGURE 1. Boussinesq problem. Stresses in a homogeneous soil deposit due to a surface loading.

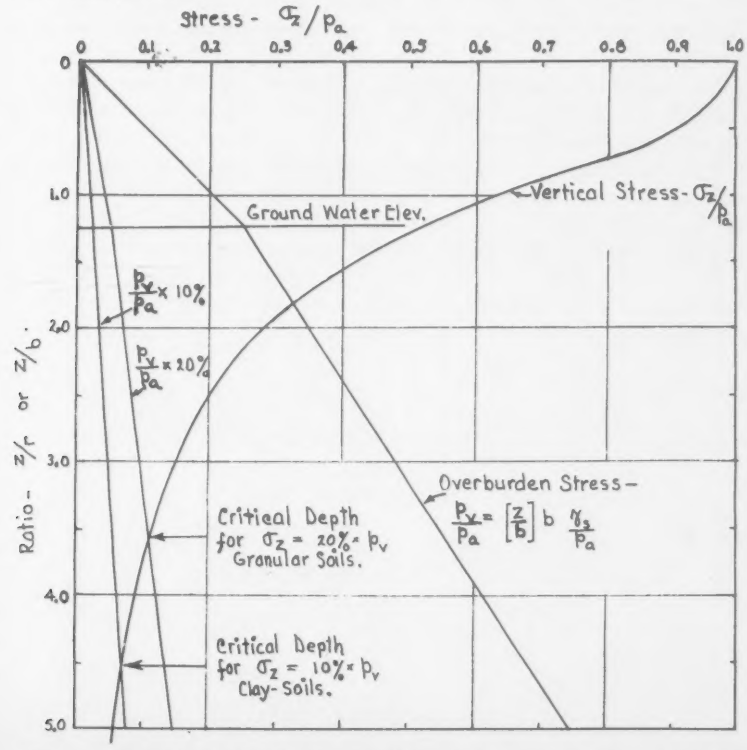

FIGURE 2. Critical depth for distributed foundation loads of structure for preliminary investigations.

tions the stress decrease is less with depth, since it is a function of the basic ratio, $^{z}/_{r}$ or $^{z}/_{b}$, and conditions are less favorable. Use-influence diagrams and tables for distributed loads have been presented by Newmark,[4] Fadum,[5] and Terzaghi.[6]

Case 2. The Westergaard problem[7] gives the vertical stress conditions imposed in the supporting soils of a thinly and uniformly bedded soil deposit beneath a uniformly distributed foundation load, applied at the surface of the ground, with this surface free of normal and with shearing stresses beyond the limits of the loaded area. This case is valid for a soil deposit which is visibly and uniformly thin-layered within the critical depths as shown in FIGURES 2 and 3. It is to be especially noted that the Westergaard distribution of vertical stresses is considerably more favorable than the Boussinesq distribution. This is due to the horizontal reinforcing effect of the thin layering and to restricted horizontal displacements thereby produced.

Case 3. Burmister's two-layer problem with a rigid base layer[8, 9] gives the vertical-stress conditions imposed in the upper compressible layer of the system beneath a uniformly distributed foundation load applied at the surface of the ground, with this surface free of normal and shearing stresses beyond the limits of the loaded area. This case is valid where the rigid base layer is within the critical depths given in FIGURES 2 and 4. The distribution of vertical stress is now governed by two basic parameters, $^{r}/_{h}$ and $^{z}/_{h}$, and by the elastic constant, Poissons ratio. The vertical-stress distribution for the simple case at the rock surface only was given first by Biot,[10] who showed a stress concentration of 150 per cent of the Boussinesq values, and later by Picketts,[11] who evaluated a few influence curves for stresses at the rock surface. While the results of the complete investigation by Burmister are not yet available, it was

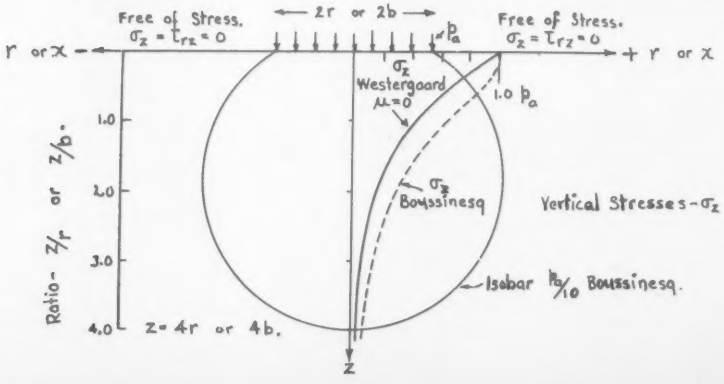

FIGURE 3. Westergaard problem. Uniformly thin-layered deposit.

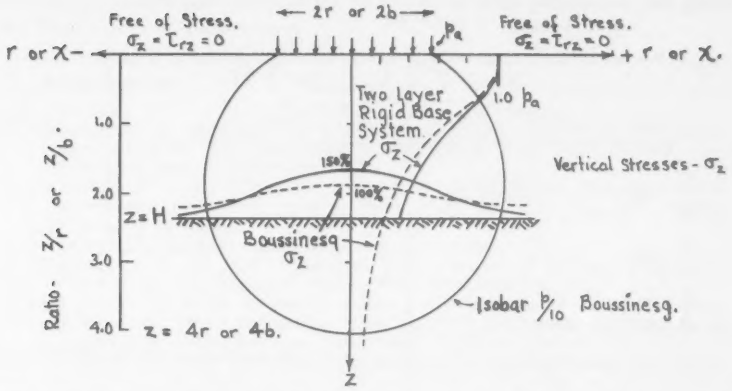

FIGURE 4. Burmister problem. Two layer system with a rigid base layer at depth, H.

found that a concentration of stress existed through the deposit as noted in FIGURE 4, which represents a less favorable stress condition than that given by the Boussinesq problem. While the rigid base layer does restrict settlement to the upper compressible stratum, account must be taken of the higher and less favorable stress conditions in this case.

Case 4. The Burmister two- and three-layer problems give the vertical-stress conditions imposed in a deposit composed of a system of soil layers within the critical depth beneath a uniformly distributed foundation load applied at the surface of the ground, with the surface free of normal, and with shearing stresses beyond the limits of the loaded area. Where a strong surface layer is underlaid by a weaker, more compressible layer, as shown in FIGURE 5, the strong layer has an important load spread and reinforcing capacity in reducing the intensities of the stresses imposed in the weaker, underlying, more compressible layer, which is the favorable and dominant characteristic of this layered system. The effectiveness of the reinforcing action of the strong layer, however, depends not only on the ratio of the strength characteristics E_1/E_2, but also, markedly, on the layered-system parameter, r/h, which is the ratio of the equivalent radius of the loaded area to the thickness of the reinforcing layer. While the results of the complete investigation by Burmister are not yet available, the curves of vertical stresses in FIGURE 6 illustrate the marked increase in reinforcing capacity of layer-1 as the strength ratio E_1/E_2 increases, and is a very favorable aspect of this two-layer system. But it should also be noted that, as the size of the loaded area increases, the effectiveness and reinforcing capacity of the two-layer system decreases through the influences of the basic layered system parameters, z/r and r/h, which is an unfavorable aspect for the larger size loaded areas. A few influence values and curves of vertical stresses covering a limited

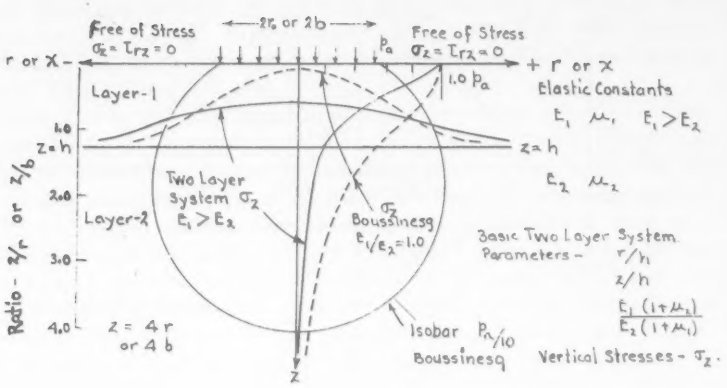

FIGURE 5. Two-layer system, strong surface layer. (Burmister.)

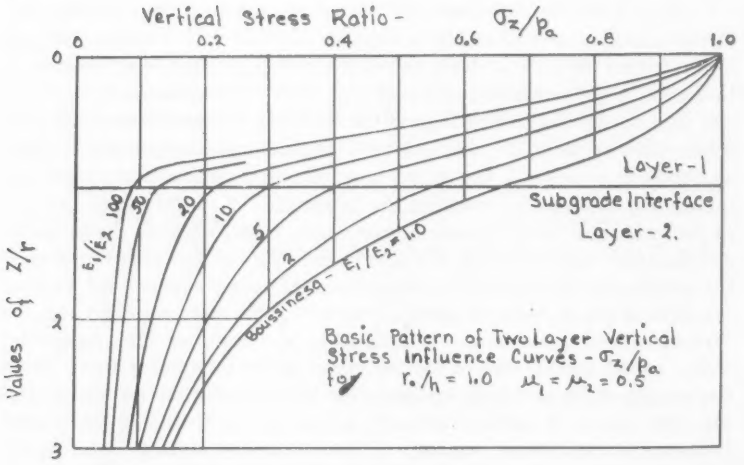

FIGURE 6. Evaluation of effectiveness of two layer systems and three layer systems in reducing vertical stresses. Burmister problem.

number of values and range of the two-layer system parameters are given by Fox.[12]

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DIVISION OF MYCOLOGY

THE GENUS *CRYPTOCOCCUS*: THE PRESENT STATUS AND CRITERIA FOR THE IDENTIFICATION OF SPECIES*

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Much has been written concerning the yeastlike fungi that produce neither true mycelium nor ascospores and consist only of a simple vegetative budding cell. The literature deals not only with their classification and nomenclature but also with their correct identification and pathogenicity for man and other animals.

The history of the nomenclature of these fungi has been adequately dealt with by Benham (1935 and 1950), Skinner (1950), and Lodder and Van Rij (1952), and will therefore be dealt with only briefly in this report. The terminology and classification as proposed by Lodder and Van Rij in their 1952 publication, *The Yeasts, a Taxonomic Study*, is now generally accepted. Since these fungi do not form ascospores, they must be placed in the Fungi Imperfecti.

Lodder and Van Rij gather these asporogenous yeasts in a separate order, termed the Cryptococcales. They recognize one family, the Cryptococcaceae, in which they place both the mycelial and nonmycelial asporogenous yeasts. There are three subfamilies, as follows: (1) Cryptococcoideae, (2) Trichosporoideae, and (3) Rhodotoruloideae. A number of genera are to be found in the Cryptococcoideae, and one each for the other two families. The genus *Cryptococcus* as now defined belongs, then, in the subfamily Cryptococcoideae. This arrangement seems to be the one preferred by students of the yeasts, both in the United States and in Europe.

The genus *Cryptococcus* is limited to the simple, spherical, or oval encapsulated organisms that reproduce by budding and do not produce mycelium or ascospores. These organisms are nonfermenters, and they produce starch under suitable conditions. Lodder and Van Rij, on the basis of size and shape of cells, and of nitrogen and carbon assimilation, recognize five species. One of these is *Cryptococcus neoformans*, the etiological agent in cryptococcosis (European blastomycosis or torulameningitis). Their study is, however, a purely taxonomic work, giving no information as to the degree of virulence or pathogenicity for man or animals.

Benham (1935), in a study dealing with the identification and classification of the nonmycelial, nonspore-forming yeastlike fungi included at that time in the genus *Cryptococcus*, divided them into four groups on the basis of colony characteristics, agglutination reactions, and sugar fermen-

*This paper, illustrated with lantern slides, was presented at a meeting of the Section on February 25, 1955.

tations. In the Lodder and Van Rij classification, these four groups represent three genera in the family Cryptococcaceae, group 1 representing the noncapsulated, fermenting organisms now placed in the genus *Torulopsis;* groups 2 and 3, representing the capsulated, nonfermenting organisms, now comprise the genus *Cryptococcus;* while group 4, representing the red pigment-producing organisms, is now placed in the genus *Rhodotorula,* of the subfamily Rhodotoruloideae.

In group 3, which now represents the genus *Cryptococcus* as defined above, Benham recognized two subgroups: (1) the pathogen, *Cryptococcus neoformans (hominis)* and (2) a nonvirulent variety which she named *C. hominis,* var. *innocuous.* These two varieties could be separated one from the other only by a virulence test and their ability to grow at 37° C. It seemed of interest, therefore, to study Lodder and Van Rij's five species from the standpoint of virulence and pathogenicity, and to re-evaluate some of the criteria now used in the identification of these simple fungi. Twelve isolates were used in this study: five representative of Lodder and Van Rij's species, six isolated in our laboratory, and a strain purporting to be the original Busse-Buschke strain.

A list of the strains included is as follows:
(1) *Cryptococcus neoformans.* (Sanfelice). Received 1953 from C.B.S.
(2) *Cryptococcus neoformans.* Isolated 1951 by Benham.
(3) *Cryptococcus neoformans.* (Busse-Buschke). Received 1934 from Ota.
(4) *Cryptococcus laurentii.* Received 1953 from C.B.S.
(5) *Cryptococcus luteolus.* Received 1953 from C.B.S.
(6) *Cryptococcus mucorugosus.* Isolated 1934 by Benham.
(7) *Cryptococcus diffluens.* Received 1953 from C.B.S.
(8) *Cryptococcus albidus.* Received 1953 from C.B.S.
(9) *Cryptococcus neoformans,* var. *innocuous.* Isolated 1934 by Benham.
(10) *Cryptococcus neoformans,* var. *innocuous.* Isolated 1949 by Benham.
(11) *Cryptococcus neoformans,* var. *innocuous.* Isolated 1953 by Benham.
(12) *Cryptococcus neoformans,* var. *innocuous.* Isolated 1950 by Benham.

Each of these strains produces a wide gelatinous capsule associated with a mucous type of growth, forms no true mycelium, and does not produce ascospores. They all form starch and all fail to ferment sugars. These characteristics assure their position in the genus.

For distinguishing the pathogenic or *Cryptococcus neoformans* from the nonpathogenic or *Cryptococcus neoformans,* var. *innocuous,* the ability to grow at 37° C. and virulence for mice was noted for each of them. The three strains labelled *C. neoformans,* namely Lodder's (Sanfelice's), the original Busse-Buschke strain, and one strain isolated in our laboratory, proved virulent for mice when injected intraperitoneally, invading and forming typical lesions in the brain, and killing a mouse in 14 to 21 days or more. These isolates were recovered from the brain and other organs at 37° C. and at room temperature. Of the remaining nine strains, six were

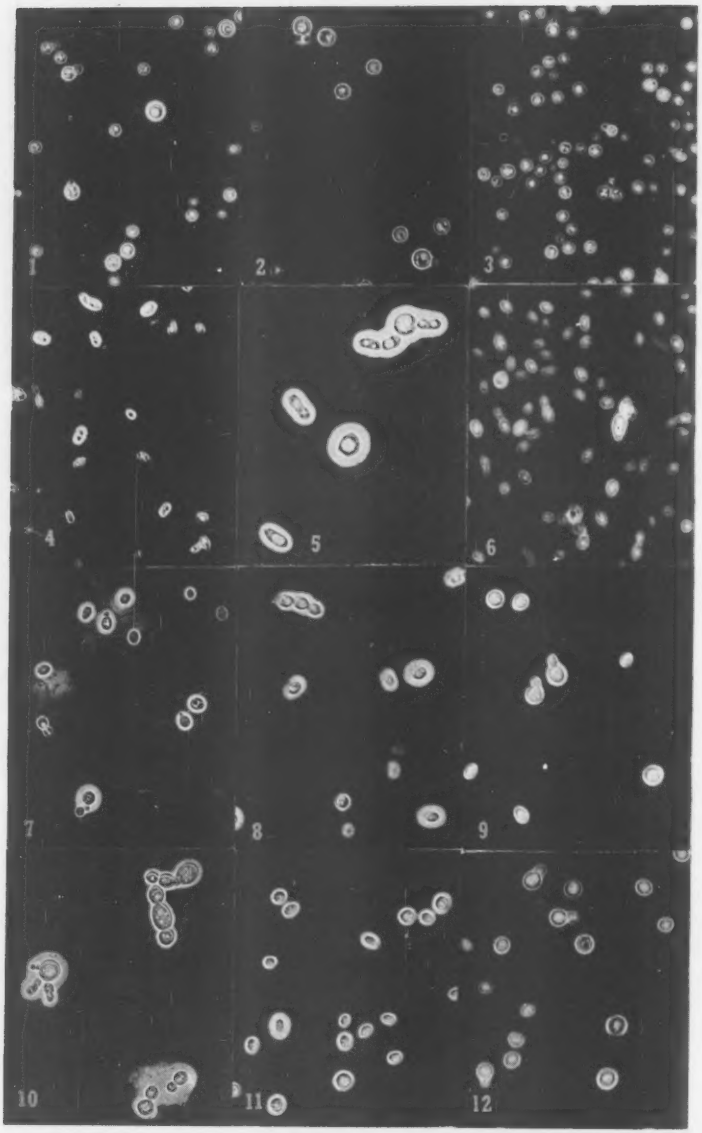

PLATE 1. Photomicrographs of each of the 12 organisms used in this study from 24-hour cultures on Sabourand's dextrose agar, mounted in India ink, × 430.

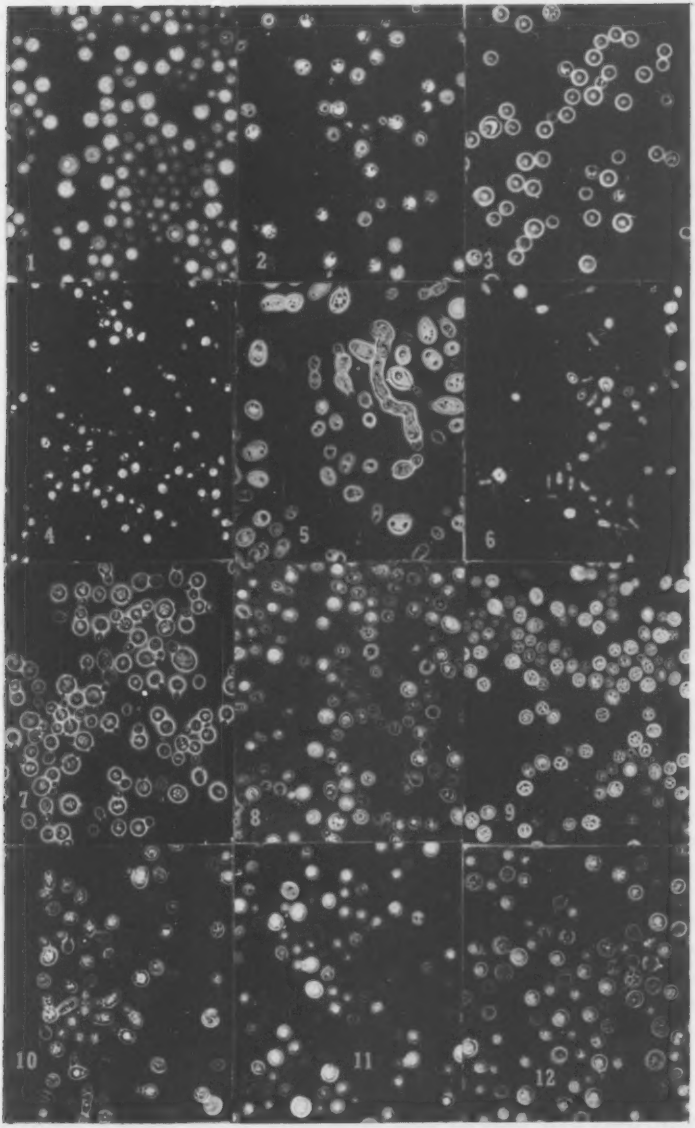

PLATE 2. Same 12 organisms as in PLATE 1 taken from one-month-old cultures on Sabouraud's dextrose agar, mounted in 10 per cent nigrosin, × 430.

nonpathogenic for mice and failed to grow at 37° C., thus falling into Benham's var. *innocuous*. Finally, two of Lodder's species, *C. laurentii* and *C. luteolus*, and Benham's *C. mucorugosus* showed slight differences from the above groups, e.g., sometimes a weak growth at 37° C., or a mild degree of virulence. These species were recovered, after 7 to 14 days, from the peritoneal cavity and never invaded the brain.

For species differentiation, as mentioned above, much importance is now placed on the ability to assimilate potassium nitrate and the carbohydrates dextrose, maltose, saccharose, lactose, and galactose. Various methods have been used in determining the ability to assimilate a given nitrogen or carbon source. These have been based chiefly on the auxanographic method that was first introduced by Beyerinck (1889) and then applied to the study of yeast taxonomy by Lodder (1934). This method, in brief, is carried out as follows: a tube of molten, basic agar medium, lacking only a source of nitrogen or carbon, as the case may be, is inoculated with the organism to be tested, then mixed and poured into a plate, and allowed to harden for several hours. A few crystals of the substances to be tested are then placed at various points on the surface of the agar near the edge of the plate. No more than three substances are used on a plate, and each plate is incubated at room temperature. Growth will take place in a zone corresponding to the diffusion of the carbon or nitrogen source whenever a compound is assimilated. Wickerham and Burton (1948) objected to the agar-plate method for assimilation tests on the basis of an inadequate basal medium and the short incubation period, limited by rapid drying out of the medium. They devised a liquid medium which proved adequate, and permitted the longer incubation period that is necessary for the successful testing of pentoses, polyhydric alcohols, and organic acids. Readings in this method are made by determining the density of growth, either in a Lumitron photometer or by placing the tube against a white card bearing India inked lines approximately ¾ mm. thick, and 5 mm. apart. If the lines are obliterated, results are recorded as 3 plus; if they appear as a diffused band, as 2 plus; if lines are distinguishable as such, but have indistinct edges, as 1 plus; if no growth, as negative. As a result of his extensive studies of the yeasts, Wickerham has prepared four basic media for the study of these fungi. These media may be obtained from the Difco Laboratories and are listed as follows: (1) yeast morphology agar; (2) yeast carbon base; (3) yeast nitrogen base, and (4) vitamin-free yeast base. The formula and directions for use are given in Wickerham's *Taxonomy of Yeasts* (1951).

In our studies, tests were made following Wickerham's method with the liquid media and using his yeast nitrogen base and yeast carbon base. Also, tests were made on solid media using, in one instance, the auxanographic technique of Beyerinck and, in the other, a new technique which we found very simple as well as very satisfactory. This new technique consisted of adding to Wickerham's yeast nitrogen base or yeast carbon

TABLE 1

ASSIMILATION OF KNO_3 BY CRYPTOCOCCI
COMPARISON OF THREE METHODS

Organism	*Liquid*	*Streak plate*	*Auxanographic plate*
1. *C. neoformans*	0	0	0
2. *C. neoformans*	0	0	0
3. *C. neoformans*	0	0	0
4. *C. laurentii*	0	0 or wk +	0
5. *C. luteolus*	0	0 or wk +	0
6. *C. mucorugosus*	+	+	+
7. *C. albidus**	+	+	+
8. *C. diffluens*	+	+	+
9. *C. neoformans* var. *innocuous*	+	+	+
10. *C. neoformans* var. *innocuous*	+	+	+
11. *C. neoformans* var. *innocuous*	+	+	+
12. *C. neoformans* var. *innocuous*	+	+	+

*Sometimes weak.

base, as the case may be, a "purified"* agar containing either KNO_3 or the sugar to be tested. The basal media were made in × 10 strength with the addition of either KNO_3 or a sugar to give the required final concentration, and were sterilized by Seitz filtering. Two ml. of the sterilized solution were placed in a petri dish, and 18 ml. of the molten and cooled 2 per cent "purified" agar solution was added, and the dish rotated to mix the solution thoroughly. When solidified, these plates were inoculated by streaking the organism in parallel streaks, beginning at the edge and working toward the center of the plate. Those organisms which gave negative results, for example, did not assimilate the KNO_3 or the particular sugar being tested, usually gave a slight growth in the first streak, due to some carry-over of nutrient with the inoculum, while the remaining streaks remained clear. The results of the studies for KNO_3 assimilation with each of the three methods outlined above are given in TABLE 1.

As may be seen in TABLE 1, the results obtained with all three methods were identical. Both the auxanographic and the streak plate have an advantage over the liquid method as they are easier to prepare and give clear-cut results in 48 hours. The streak plate is simpler than the auxanographic plate and is to be preferred for preliminary tests. If a slight growth appears, or if the results seem doubtful, an auxanographic plate should be used. In strictly taxonomic studies where a large number of compounds

*Three types of "purified" agar were used, all giving equally satisfactory results: (1) agar purified by the Robbins method;[4] (2) "washed" agar, described by Lodder and Van Rij, 1951; (3) special agar, Noble, a Difco product. "Washed" agar is recommended as it is cheapest and easiest to prepare.

are used and a longer incubation period needed, Wickerham's liquid medium would be of an advantage. In identifying species commonly found associated with man, however, and in differentiating virulent from nonvirulent strains of *Cryptococci*, the agar-plate method is, in our opinion, preferable.

On the basis of KNO_3 assimilations, three groups may be differentiated:

(1) Those which do not assimilate KNO_3. The three *C. neoformans* strains fall into this group.

(2) Those which do assimilate KNO_3. These represent the var. *innocuous* strains and Lodder's species *C. diffluens* and *C. albidus*.

(3) Those which give a weak or negative assimilation of KNO_3. These represent Lodder's species, *C. laurentii* and *C. luteolus*.

As to the carbon assimilation (TABLE 2), all the isolates studied utilized dextrose, maltose, and saccharose, but only Lodder and Van Rij's species *C. laurentii* and Benham's *mucorugosus* assimilated lactose. All three strains of *C. neoformans* were negative for this sugar. The pathogenic strains and *C. laurentii* and *C. luteolus* were capable of assimilating galactose. It will be noted, in this respect, that these same two species gave a weak assimilation of KNO_3, thus showing a closer relationship with *Cryptococcus neoformans*. The species *C. mucorugosus* likewise gave a definite assimilation of lactose and a weak assimilation of galactose, suggesting that, upon further study, this species and some of our *innocuous* strains might be found identical with, or closely related to, Lodder and Van Rij's nonpathogenic species *albidus*, *diffluens*, and *luteolus*.

TABLE 2

ASSIMILATION OF CARBOHYDRATES BY CRYPTOCOCCI
RESULTS WITH FIVE SUGARS

	Dextrose	*Maltose*	*Saccharose*	*Lactose*	*Galactose*
1. *C. neoformans*	+	+	+	0	+
2. *C. neoformans*	+	+	+	0	+
3. *C. neoformans*	+	+	+	0	+
4. *C. laurentii*	+	+	+	+	+
5. *C. luteolus*	+	+	+	0	+
6. *C. mucorugosus*	+	+	+	+	+
7. *C. albidus*	+	+	+	0	0
8. *C. diffluens*	+	+	+	0	0
9. *C. neoformans* var. *innocuous*	+	+	+	0	0
10. *C. neoformans* var. *innocuous*	+	+	+	0	0
11. *C. neoformans* var. *innocuous*	+	+	+	0	0
12. *C. neoformans* var. *innocuous*	+	+	+	0	0

Since Lodder and Van Rij include size and shape of cells in the criteria for distinguishing species, it would seem necessary to discuss these factors as well as the appearance of the macroscopic growth of the above 12 strains. The character of the macroscopic growth varies somewhat with the medium and, in this study, was noted on Sabouraud's dextrose agar, malt extract agar, and Wickerham's morphology agar. In general, some clue as to whether a given isolate is *Cryptococcus neoformans*, or a nonvirulent variety, may be obtained from the macroscopic growth. Briefly, *C. neoformans* on Sabouraud's dextrose grows somewhat heavier than the nonvirulent strains, forming a more or less dry cream to orange-tan growth. The innocuous strains produce a moist, glistening mucoid growth, some a faint pink in color, others a gray-tan. The intermediate group contains three more or less distinct species: Benham's *C. mucorugosus* and Lodder and Van Rij's species, *C. laurentii* and *C. luteolus*. This group gives three distinct types of growth: *C. mucorugosus*, a somewhat wrinkled colony with a tan pigment; *C. laurentii*, a dull, raised, orange-tan growth; and *C. luteolus*, a lemon yellow, thin-flowing type of growth. On Wickerham's morphology agar, a more marked differentiation in pigment is noted. The pathogen *C. neoformans* produces, at first, little pigment, and only a moist cream-colored growth. As growth continues, an orange-tan pigment forms, and when the colony is about three weeks old it assumes a deep orange-tan color. On the other hand, the *innocuous* strains produce in the same manner a pale, pink pigment, not, however, to be confused with the pigment of the Rhodotorulas, which is much deeper. The appearance of the three distinct species has already been mentioned. On malt-extract agar, a more mucoid type of growth with a deep tan pigment was produced by most strains. The species *C. laurentii* produced a dull, dry, spreading growth.

The differences noted in macroscopic growth are more or less borne out by microscopic appearances. Observations were made and photomicrographs taken (all at the same magnification) of 24-hour Sabouraud dextrose agar cultures. The three *C. neoformans* strains show a surprisingly small capsule, which is rather usual for this species, on this medium. The cells are typically round, of varying diameter, ranging from 3 to 4 and 5 to 8 μ without capsule. This range in size may occur within a given isolate, or different isolates may vary one from another in the average size. For example, the *C. neoformans* strain from Lodder appears smaller than the other two strains. The nonvirulent or innocuous strains show fairly uniform spherical or oval cells with rather wide capsules. This characteristic undoubtedly tends to furnish the more mucoid type of growth.

The three strains which appear to represent distinct species, namely, *C. laurentii*, *C. luteolus*, and *C. mucorugosus*, show definite morphological differences. *C. laurentii* has a small oval cell, usually with two prominent granules, one at each end of the cell. The budding cells have fine granules with the capsule absent or very narrow. *C. luteolus*, on the other hand, produces a large budding cell, often elongated and with an unusually wide

TABLE 3

SUMMARY OF RESULTS OF STUDY WITH 12 STRAINS OF CRYPTOCOCCI

Organism	Growth at 37°	Path. for mice	KNO_3 assim.	Carbon assimilation Dextrose	Maltose	Saccharose	Lactose	Galactose
1. *C. neoformans*	+	+	0	+	+	+	0	+
2. *C. neoformans*	+	+	0	+	+	+	0	+
3. *C. neoformans*	+	+	0	+	+	+	0	+
4. *C. laurentii*	0	0	0	+	+	+	+	+
5. *C. luteolus*	0	0	0	+	+	+	0	+
6. *C. mucorugosus*	0	0	+	+	+	+	+	+
7. *C. albidus*	0	0	+	+	+	+	0	0
8. *C. diffluens*	0	0	+	+	+	+	0	0
9. *C. neoformans* var. *innocuous*	0	0	+	+	+	+	0	0
10. *C. neoformans* var. *innocuous*	0	0	+	+	+	+	0	0
11. *C. neoformans* var. *innocuous*	0	0	+	+	+	+	0	0
12. *C. neoformans* var. *innocuous*	0	0	+	+	+	+	0	0

TABLE 4

DIFFERENTIAL CHARACTERISTICS OF THE CRYPTOCOCCI
VIRULENT VERSUS NONVIRULENT

Group	Growth at 37°	Path. for mice	KNO_3 assim.	Carbon assimilation: Dextrose	Maltose	Saccharose	Lactose	Galactose
(1) *C. neoformans* 17 strains	+	+	0	+	+	+	0	+
(2) *C. neoformans* var. *innocuous* 7 strains; *C. albidus* 1 strain; *C. diffluens* 1 strain	0	0	+	+	+	+	0	0
(3) *C. laurentii*	0	±	0 or wk.	+	+	+	+ wk.	+ wk.
(3) *C. luteolus*	0	±	0 or wk.	+	+	+	0 or wk.	+
(3) *C. mucorugosus* 1 strain of each	0	±	0 or wk.	+	+	+	+ or wk.	+ wk.

capsule. The buds tend to remain attached, forming a rudimentary pseudomycelium. *Cryptococcus mucorugosus*, the one species from Benham's group 2, has elongated cells and a narrow capsule. A similar study was made of one-month-old cultures on Sabouraud's dextrose agar and differences were again noted. The *C. neoformans* strains are more truly round and more highly granular than the var. *innocuous* strains. All show a number of thick-walled cells. Some show the peculiar thick-walled triangular cell containing a single body near the apex, where the wall is thinner. This structure has been considered by some to be an ascus with an ascospore; by others, as a modified capsule. There is, however, much doubt as to the ascus nature of this structure. It is very unlike ascospore formation as seen in other yeasts. Also, similar thick-walled cells are noted in old cultures of many yeasts. A recently isolated strain produced, after three months on Sabouraud's dextrose agar, an exaggerated cell of this type, with the wall piled up in layers on two sides of the cell. All stages of shedding of these outer layers were seen. This phenomenon needs further study.

In TABLE 3 the complete results with the 12 strains included in this study are summarized. In addition, 14 more strains of *C. neoformans* have been screened and found to conform in every respect with the three strains included in TABLE 3. The 14 additional strains include five isolated from the soil; six from bovine mastitis, received from Doctor Emmons; a recent isolate from our laboratory; and two from spontaneous cases (one in a cat, the other in a dog), received from Doctor Hazen of the New York State Public Health Laboratories. Three additional strains of the nonvirulent type isolated in our laboratory were also screened and agreed with our findings for the innocuous group.

In TABLE 4, the criteria which distinguish the three groups of *Cryptococci* are listed, based on virulence and associated characteristics.

The results of these studies have confirmed the opinions of Lodder and Van Rij, and of Wickerham in showing that KNO_3 assimilation is an aid in identifying species of *Cryptococci*. Further, this work has shown that the test is valuable in distinguishing virulent from nonvirulent cryptococci and that nonassimilation of nitrates should be added to the ability of the organism to grow 37° C. and the virulence for mice, as diagnostic characteristics of *Cryptococcus neoformans*. Carbohydrate assimilation, even when limited to the five sugars used in this study, is a real aid in distinguishing species and, it would seem, in distinguishing virulent from nonvirulent varieties, with the carbohydrate glactose being especially important in this respect.

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COMMUNICATION AND SOCIAL CHANGE, BASED ON A FIELD STUDY IN NORTHERN RHODESIA*

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In many parts of Africa, of which Northern Rhodesia is one, members of former preliterate, preindustrial rural societies are literally leaping through centuries of change in a few decades. Some idea of this change may be gained from the following illustration. One evening, about six o'clock, when I was driving in the African compound,** I passed the following activities in about 20 minutes: (1) a meeting of the African Mine Workers Union in the movie amphitheatre of the Mine Welfare Hall; (2) a rehearsal for a tribal dance to be held the following Sunday afternoon; (3) the showing, by a missionary, of a silent film of Noah's life to the drum beats of the nearby dance rehearsal. Had these events not been occurring simultaneously, some individuals would probably have attended all three of them. A fascinating part of this community, for me, was the manner in which the old, the partly old, and the new existed side by side and even achieved a partial integration.

The mining community in which I worked is on the Copper Belt of Northern Rhodesia and had a population of approximately 50,000 Africans, 5,000 Europeans, and a few hundred Indians. The Africans represented 90-odd Bantu tribes, but these could be conveniently classified into 10 or 12 groups, based on their area of origin. Bemba was the *lingua franca*. The Europeans, the majority of whom were employed by the mine, were mostly British–English, Scotch, Irish, Southern Rhodesian, South African, Canadian, etc. There were a sprinkling of refugees from Central and Eastern Europe and an occasional American. This is the setting in which the study was made.***

I was interested in exploring problems concerned with the communication of European culture to Africans, which, of course, is one way of studying social change. Communication involves the communication of symbols and the integration of old symbols with new ones, which is a mechanism of social change, whether in the technical or moral orders. As Robert Redfield has so clearly pointed out, the expansion of the techni-

*This paper is a condensation of the one presented at a meeting of the Section on February 28, 1955. The field work on which it is based was carried on in Northern Rhodesia (1953-1954) by the author while on sabbatical leave from Queens College, Flushing, N. Y., and was supported by grants from the Guggenheim Foundation, New York, N. Y.

**The term "township" has recently been introduced because it denotes a higher status than "compound," but is not heard very often colloquially.

***Unless otherwise indicated, the words, "African" and "European" refer in this paper only to members of the area studied.

cal order comes first in the development of civilization, but the resulting changes in the moral order are far-reaching and underlie civilization's continuing development. In the moral order, men are bound together by what they consider right or good, while in the technical order, men are bound by things and organized by necessity or expediency.[1] This study was concerned primarily with changes in the moral order, and the core of these changes lies in modifications in the value systems. The African moral order of the past, like those of other preliterate societies, was understood by all, but was implicit. Today, the new order is not yet fully comprehended and is explicit rather than implicit. Neither the new nor the old moral system covers the whole round of life for any African, whether he is oriented along traditional or modern lines. The two orders operate simultaneously, sometimes in a harmoniously integrated manner, and sometimes in conflict.

Any moral order, traditional or new, has to be communicated, and the communication of such an order is far more complicated than that of a technology. The mechanisms of communication underlying a class in which miners are taught how to use new tools are relatively simple and direct. The introduction of any part of a new moral system is, on the other hand, a complicated, slow, and long process.

To understand this process, I began with Harold Lasswell's now famous formula of "Who, says what, to whom, under what circumstances, and with what effect." I use the following axioms which show how responses to symbols of communication depend, to a large degree, upon certain psychological and sociological characteristics of the audience:

(1) *Their imagery of self and of others.*[2] These images arise out of experiences, some genuine (events in the environment) and others fostered (propaganda). Most images are composite, containing benevolent and malevolent features, but one of the two aspects usually predominates over the other.

(2) *Lasswell's principle of maximization of values.*[3] What "cues" are perceived and how they are interpreted depends on whether values are maximized or threatened.

(3) *New and old symbols.* New symbols tend to find some level of integration with old ones.

The specific content of the communication, of course, is not unrelated to the response, and I would extend the above postulates to the communicator. What he communicates will depend on *his* images and *his* values, and they too will be related to his experiences.

This paper deals with only a very small part of the communication between Europeans and Africans.* It is limited to: (1) Briefly describing

*The larger research project was concerned with responses to symbols of communication on the impersonal level through the mass media (radio, movies, and press) and on the personal level through interpersonal relations. Data are of both a quantitative and qualitative nature, but for this paper the data is primarily qualitative.

changes in the "who's" and in the circumstances (political, economic, and social environment), and showing how these changes threaten two basic values of Africans; (2) Showing how the resulting imagery of Europeans held by Africans, based on reality, has also, in some cases, become tied in with traditional ways of magical thinking, which gives the imagery a special intensity; and (3) Indicating how points 1 and 2 account for a certain predisposition among Africans to some of the symbols of European communication.

The first "who's" representing European culture were Doctor Livingstone and the missionaries who followed him. The first permanent mission station was established in Northern Rhodesia in 1885. Four years later, the British South Africa Company was given power to administer the territory and to engage in trade. In 1924, the country became a Protectorate under the British Crown, administered by civil servants. The development of Northern Rhodesia's copper industry began about 1925 and, after the world depression in the early 1930's, developed rapidly and now dominates the economy of the Territory.[4] Very recently (August 1, 1953), Northern Rhodesia became part of a new Federation with Southern Rhodesia and Nyasaland. Each political change represents a change in the locus of power between the various European groups and in their relative number. The new Federation represented an increase in power for the European settlers, who have also become more numerous, and a loss of power for the British Colonial Office. Africans throughout the whole territory have vigorously but unsuccessfully protested the coming of the new Federation, because they apparently have greater confidence in the Colonial Office than they have in the European settlers.

The Europeans in the "who's" of our formula can be classified into different groups according to their value systems. The missionaries were, and still are, to a large degree, dedicated men. They are dedicated to helping the Africans achieve the Christian moral order and are the type of men who try to live out their belief that all men are the children of God. Many of the early civil servants were also, and still are, to a large degree, dedicated men. Their goal was to bring Africans to the European level of civilization, and African interests were considered paramount.[5] Missionaries and civil servants were primarily in Africa to help Africans. But "home" for these Europeans was still Great Britain, to which they went "on leave" and, usually upon retirement.

In the old days, many of these Europeans knew the Africans of their district personally. Some, usually among traders, took African concubines and, occasionally, an African wife. For many, a pastime was to go hunting with Africans and, at the end of a day's hunt, Europeans and Africans would sit around the fire together feasting on the spoils. In talking to Europeans who have been in the territory 25 years or more, I

received the very strong impression that they had indeed enjoyed their life in the "bush." The district commissioners and officers were stern men, but with the traditional concept of British justice. Moveover, they were not cruel, as were so many of the African chiefs. Tales are still told of how an African chief thought nothing of cutting off a hand of a person caught in adultery with one of his wives. From the reality of experience, Africans had an image of Europeans (mostly missionaries and civil servants) which took the form of a benevolent father leading and teaching his African children the ways of European civilization.

This image appears frequently in the verbatim recordings of the early meetings of the African Representative Council. At one of the meetings, in 1947, during the discussion of a motion to prevent Indian immigration, frequent comparisons between Indians and Europeans were made by the African discussants. One said, "The only thing they (the Indians) do is to cheat us. We speak well of the Europeans because they have taught us many things. They have turned our people into teachers, they have taught us woodwork, they have taught us to become builders and so on, and they have taught us the way of knowing God. They have made us what we are today."[6] In the same context, another speaker said, "Our missionaries lost their lives in nights of darkness and in days of darkness. Our native commissioners and our district officers have borne the burden of civilizing the Africans in this country...."[7] On another day, the discussion was on a motion to increase the number of European representatives of African interests in Legislative Council, and the maker of the motion ended his oration with, "...and they (the people whom he represented) want the government to listen to their cries. The government is our father."[8]

There were also, of course, unfavorable elements in the African's image of the missionaries and civil servants but, on the whole, it appears to have been a benevolent one. The missionaries and civil servants are still present, but they have not that close personal contact that prevailed in the past with large numbers of Africans. Particularly is this true of the civil servants, who have to spend most of their time in offices, bowed down with paper work and administrative detail, and have neither the time nor the opportunity to know more than a relatively few Africans. They are also being increasingly superseded by the European settlers, who include top industrialists and those in the managerial class, miners and other workers, shop keepers and their employees, and professionals, such as doctors, engineers, etc. These settlers are like any other colonists immigrating anywhere in the world. They obviously do not come to Northern Rhodesia to help the Africans. They come to make a living and to establish a home for themselves and for their children and grandchildren. For many, immigration means raising their economic standards, and living in a style to which they are *not* accustomed. For others, it

may mean maintaining a style of life no longer possible in England. "Home" for most of them (except those at the top levels) is Northern Rhodesia. They do not agree with the concept of "Paramountcy of Native Interests," but they think that the interests of those who are developing the natural resources of the country should come first. The settlers' attitude appears to represent a different theory of colonialism than that held by the British government.

The image of the African as a child who should be taught the ways of European civilization and who eventually would become Europeanized in culture is now being replaced by a many sided picture. For the top level industrialist and for management, the African is a necessary and useful worker in the exploitation of the natural resources and, in general, their policy, based on enlightened self-interest, is one of gradual economic advancement for him. When the European mine worker considers the African, he appears to hold two conflicting views—not unrelated in causation—simultaneously. On the one hand, the African miner is greatly feared as a potential rival for the jobs now held by Europeans and, on the other hand, the same European individual will refer to him as a "monkey just down from the trees," and incapable of taking on European civilization. The African's lack of technological inventions, such as automobiles and airplanes, is regarded by these Europeans as a proof of the African's innate inferiority. At the same time, the Africans are becoming increasingly inducted into European civilization through schools, the mass media, employment in industry, *etc.* A new middle class is emerging, for whom a major goal is the ownership of a shop or "tea-room." Many of the European middle-class men who own shops feel threatened by the increasing rivalry for a growing African trade. On the political side, there is a widespread fear of African potential political power based on their preponderance of numbers. The West African political development arouses apprehension. For the African, it is the example of South Africa which causes anxiety. It is at least possible to conceive of the new Federation hewing out its own future, based neither on the example of West or South Africa.

I have briefly traced, in an altering political and economic environment, some of the changes in the European's imagery of Africans. These have been communicated subtly and directly to the Africans in many ways. In essays written by Africans, they often refer to the way Europeans speak of them as monkeys. Changes in the European's imagery of Africans has been accompanied by related changes in the African's imagery of Europeans. An African referring to a missionary said, "He is not a European; he is a missionary."

The African's new imagery of Europeans appear to have been caused by a fusion of (1) the actual changing character of the Europeans and the African's knowledge of the European's imagery of him; (2) the political event of Federation; and (3) the impact of points 1 and 2 on two

basic and related African values—land and health.* The possession of land was, and still is, one of the most important values. The background of these people is agricultural and pastoral, and land appears to be synonymous with life. It should be remembered that Africans, like other preliterate peoples, have considered themselves as part of nature rather than outside it. In the past, the value of land seems to have been largely implicit and taken for granted. Today, it is explicit and there is an intense fear of losing the land because of the Federation. This theme ran through all the discussions of the African Representative Council when they were talking on the subject of Federation and is reflected today in the casual conversations of many Africans.

The following are quite typical speeches made by members of the African Representative Council during discussions of the proposed Federation or, as it was sometimes called in the beginning, "self-government." They illustrate past attitudes to Colonial civil servants and current attitudes to the new Federation.

"...We are strongly opposed to the proposals for self-government, because we believe these proposals are only a hoax. The truth is that the Colonial Office policy that the interests of Africans should be paramount frightens our European settlers. They are trying to drag us to the south where our interests would remain inferior forever....I cannot go into the details about the hardships which the Africans are experiencing as far as land questions are concerned both in Southern Rhodesia and the Union of South Africa....Since the motive behind the demand for self-government is to amalgamate or federate with Southern Rhodesia, a country whose native policy is one of economic and political discrimination and racial segregation, and since Southern Rhodesia is a white man's country, the whole issue, however well intended is absolutely unacceptable. Northern Rhodesia should remain under Colonial Office rule until such time as the Africans themselves begin to demand self-government."[9]

"We know that these Europeans who are today in Southern Rhodesia are looking to this country and want this country....Our present Government is trying to bring us along like children who are learning to walk, and we are just beginning to see the light and to have a little wisdom, but those who are looking to this country say 'When those young children grow up, we shall not be able to do the things we want to do in that country.' For these reasons my people have asked me to come here and say that they do not want this new constitution. They do not want it at all. We want our Government to go along with us. When the day comes when we realize that we are fully developed, we shall be able to go forward and say, 'Father, we are now grown up, cannot we do this?'

*Another significant factor—for which there is not time for discussion now—is the changing self-imagery of the African, due to his increasing European education and use of the mass media, and his knowledge of, and contacts with, other parts of Africa.

That will be the time when we shall realize that we have in fact grown up. Now, these people who have proposed self-government have something which they want to bring to this country. There is good, fertile land here, we have wells underneath the ground, and the time will come when we will be able to develop our land and use what we have in the ground. We should not let other people have these things. These people say that if they do not get these things now, then they will never be able to get them in the future."[10]

"We are quite determined in rejecting federal government because we are well aware of the attitude of most Europeans toward the African people in this country. There is no doubt about this. It is shown in post offices, banks, and the shops when an African is not served as a human being. Before we receive the proposals on a closer association we must be shown a better attitude by the European community....Gentlemen, I think you will all agree with me that every African in this Territory respects our Europeans more than he respects his own chief. Never mind that he be a poor gentleman or he may be a little boy. Now, the Africans are beginning to say that they have respected the Europeans too much. I think most of the elder people here will agree with me, in our day we trusted our officials very well indeed because they did what they could for the indigenous people. Now an African sees a great difference and assumes that the officials are getting tired of their responsibilities and therefore want to hand over the poor Africans to a government which will not do justice to the Africans."[11]

In spite of speeches, motions, and protests, Northern Rhodesia, Nyasaland, and Southern Rhodesia became a Federation. When I began my field study I was unaware of the significance of Federation. Having come from the United States, I did not know very much about politics in Central Africa. But their importance and meaning for the Africans became apparent to me when the fear of Federation and the loss of land was dragged into almost every interview and conversation, regardless of context or relevancy. For instance, after an hour or more of discussion with an educated and particularly intelligent African about the influence of movies, he ended, apropos of nothing, "Ah well, Federation is here and our land will be taken, and we shall soon be wandering around in the bush like pigs." I never interviewed on the subject of Federation, but most Africans, with or without European education, appeared to have a compulsive need to talk about it and the related fear of losing their land.

There is very likely a high level of reality to this fear, although no native land has been alienated under the new Federation, and the native land policies for Northern Rhodesia are still under the jurisdiction of the British Crown. But Africans are afraid of the future, and point to Southern Rhodesia, a member of the new Federation, and to South Africa. Both had a greater degree of self-government and independence from the

Colonial Office than the Protectorate of Northern Rhodesia, and in both the African lost land and privileges. The African therefore concludes that the new Federation which enhances the power of the European settler and reduces that of the Colonial Office will not be good for him. It is part of logical thinking to predict the future on the basis of knowledge of the past. What the future will actually bring is difficult to know with certainty. Whether or not the African's prediction will be borne out by events does not affect his attitude now. Attitudes are largely determined by what people believe will happen.

The loss of land appears to be a symbol for annihilation, and this gives the African's attitude a special intensity. The relationship between land and life have a long and complicated history and is probably not confined to the conscious level. Health, well-being, and life itself, I assume, have always been highly prized values. Today, there is great stress in schools, radio programs, and the press on European instrumental values such as cleanliness, sanitation, and diet as means of achieving health and longevity. Africans frequently refer in conversation to the fact that European babies are larger and stronger than their African counterparts and to the general fact of the European's better health and longer life span. In discussions, interviews, and essays concerned with attitudes towards European education for African women, reactions were mixed. But the reason given by most of the African men who favored this innovation was that educated girls know more than uneducated ones about cleanliness and diet, and that therefore their husbands and children would probably live longer. The European instrumental values (cleanliness and diet) are being accepted, because they maximize the African's goal of long life and health.

Side by side with these European ideas about health exists the fear of witchcraft as a destroyer of health and life. The traditional belief in witchcraft persists with great strength and may even have increased.[12] It is an exceptional African who has not retained some belief in witchcraft. It is far easier to learn English than a new theory of causation. The scientific theory of natural and impersonal causation makes but slow headway in replacing the African one of personal and supernatural causation. As in the case of most preliterate societies coming into contact with modern ones, belief in the white man's medicines and the traditional belief in the native magic of health, sickness, and death exist side by side for a long time.

It is perhaps not too surprising that the African's image of the European, who has brought into being the new Federation—symbol for loss of land and annihilation—has become tied in with the still prevailing witchcraft thinking. The following incident, which happened while the Africans were opposing Federation is an example:

Circulars stating that Europeans had poisoned the sugar to make African men impotent and African women sterile were secretly printed

and distributed by Africans. For a considerable time afterwards, although Africans love sugar, they did not eat it. A similar tale was circulated that a certain kind of canned food consumed by Africans had been poisoned by the Europeans. These tales were propaganda, and are part of what we have called fostered experience. But the propaganda was believed.

A hypothesis to account for its effectiveness is the strong parallelism in these and similar tales to traditional African witchcraft thinking. Witchcraft is usually employed by someone envious of another's possessions. A basic element of traditional African witchcraft is the making of "poison" to kill the victim. (There are many other elements in witchcraft thinking, but we are here concerned only with two.) Now, Europeans are presumed to envy the Africans for their land and to want it. Europeans have remarkable powers and knowledge, some of which are not comprehensible to the African. The European image appears, in certain situations, to have become part of the African supernatural cosmos. This has probably always been true to some degree, as seen in a folk tale of an early European being mistaken for a god, and in the belief that European medicine is the white man's magic. But the important point is a change from a benevolent to a malevolent characteristic of supernatural power. The more or less realistic and quite logical fear of the Europeans and of the new Federation has become linked with annihilation and with witchcraft thinking. This linking of rational with magical fears may account for the great intensity and pervasiveness of the fear of European malevolency. There is a tendency to perceive "cues" of this fear in situations which really have no connection with Federation. For instance, in a showing of the British Paramount News in a movie program, there was a scene of a flood in Holland and of people being evacuated from their homes. A reaction to this was: "Oh, now those Europeans will come here and take our land for their homes." Government programs for conservation of soil, of trees, of fishes, *etc.*, are often regarded skeptically by Africans. Part of this skepticism is due to unfamiliarity with the idea of planning for the future. But some, who do understand, are hostile to the program because they seem to think it is designed for the eventual use of their natural resources by the Europeans. When I first arrived, I had to break down the suspicion that I was there to implement Federation. I was white; and so, the Africans reasoned, I was a European, therefore I was out to help impose the dreaded Federation leading to annihilation. A soap company attempted to put on a sales campaign for a strong soap which was already being sold to Africans. The campaign failed because the rumor went around that use of the soap would cause them to like Federation.

This type of response is only one part of the story of communication between Europeans and Africans.* There are still many "good" Euro-

*On a quantitative level, communication between Africans and Europeans has probably increased but, as we have indicated, the quality of it in interpersonal relations has deteriorated

peans among missionaries, district commissioners and officers, supervisors and employers, doctors, *etc.* There are many symbols of European culture to which response is favorable. These symbols occur particularly in the areas of a money economy and of a raised standard of living, in European education, in an extended range of interpersonal relations, in the Christian religion, in the secularization of entertainment (sports, radio, movies, social dancing), manners, and in other fields.

This paper has been concerned with a negative aspect of the communication process. I have described how a real change in the "who's" of the formula "Who, says what, to whom, under what circumstances and with what effect" has resulted in a changed imagery of the European for the African. The image of a benevolent father leading his children to civilization, in certain situations, has become an image of a powerful rival and malevolent human being, sometimes with even supernatural power, who wants the land of the Africans. This image has behind it the reality of experience: the character of the new European settlers and their values, as well as the political event of Federation with Southern Rhodesia, which reduces the power of the benevolently inclined Colonial Office and increases that of the European settlers. The African's image of the European in the everyday world of reality appears to have entered also the supernatural cosmos and to have become fused with witchcraft thinking. This fusion, as we have previously indicated, is not completely new. The significant point is a change in emphasis from benevolent to malevolent features in both the natural everyday cosmos and the supernatural one. The actual and increasing powers of the European settlers are feared, and this fear is in the realm of logical thinking. In addition, the image of these Europeans has taken on some characteristics of the always-dreaded African witches. This thinking that fuses reality with witchcraft may account for some of the great emotional intensity and pervasiveness of the anxiety concerning Federation. The hypothesis is that this new imagery may predispose the African not only to respond with anxiety to symbols of Federation, but also to other European symbols unconnected with that political event.*

References

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*It would be of interest to study the intense anxiety of the European miner over the potential rivalry of the African. This rivalry may also be based on a combination of many elements, some known and in the world of economic reality, and others unknown, and of a psychological nature.

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CONFERENCES HELD

SECTION OF BIOLOGY

RESERPINE IN THE TREATMENT OF NEUROPSYCHIATRIC, NEUROLOGICAL, AND RELATED CLINICAL PROBLEMS

Thursday, February 3 and
Friday, February 4, 1955

Conference Co-Chairmen:
Fredrick F. Yonkman, Frank L. Mohr, and Jock L. Graeme,
Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

THURSDAY, FEBRUARY 3

Session Chairman: Francis J. Braceland
The Institute of Living, Hartford, Conn.

9:30 A.M. -

"Opening Remarks" - Hilory Koprowski, Chairman, Section of Biology, The New York Academy of Sciences; Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

"Significance of Selected Central Mechanisms for the Analysis of the Action of Reserpine" - H. J. Bein, Research Department, Ciba Limited, Basle, Switzerland.

"Neuropharmacological Aspects of Reserpine (Serpasil)" - J. A. Schneider, A. J. Plummer, A. E. Earl, and R. Gaunt, Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

"A Comparison of Effects of Reserpine (Serpasil) and Some Barbiturates on the Electrical Activity of Cortical and Subcortical Structures in the Brain of Rabbits" - Franco Rinaldi and Harold E. Himwich, Galesburg State Research Hospital, Galesburg, Ill.

"The Effects of Reserpine (Serpasil) on Emotional Behavior of Normal and Brain Operated Monkeys" - Lawrence Weiskrantz and William W. Wilson, Jr., The Institute of Living, Hartford, Conn.

"Comparison of Cortical and Subcortical Brain Waves in Normal, Barbiturate, and Reserpine (Serpasil) Sleep" - Russell R. Monroe, Robert G. Heath, Walter Mickle, and William Miller, Tulane University School of Medicine, New Orleans, La.

"The Use of Reserpine (Serpasil) in Ambulatory and Hospitalized Geriatric Psychotics" - Anthony Sainz, Veterans Administration Hospital and Iowa Psychopathic Hospital, Iowa City, Iowa.

"Observations on the Effects of Parenteral Reserpine (Serpasil) on Cerebral Blood Flow, Oxygen and Glucose Metabolism, and Electroencephalograms of Patients with Essential Hypertension" - J. H. Hafkenschiel, A. M. Sellers, G. A. King, and M. M. Thorner, Hospital of the University of Pennsylvania, Philadelphia, Pa.

Session Chairman: Nolan D. C. Lewis
The New Jersey Neuro-Psychiatric Institute, Princeton, N. J.

2:00 P.M. -

"Use of Reserpine (Serpasil) in a Neuro-psychiatric Hospital"- Nathan S. Kline and A. M. Stanley, Rockland State Hospital, Orangeburg, N. Y.

"Treatment of Chronic Schizophrenic Reactions with Reserpine"- Leo E. Hollister, George Krieger, Alan Kringel, Max I. Silver, and Richard H. Roberts, Veterans Administration Hospital, Palo Alto, Calif.

"The Use of Reserpine (Serpasil) in Shock-Resistant and Shock-Reversible Patients" - Dean C. Tasher, Manteno State Hospital, Manteno, Ill.

"The Control of Deviant Behavior in Chronically Disturbed Psychotic Patients by the Oral Administration of Reserpine (Serpasil)" - Brian C. Campden-Main and Zygmunt Wegielski, Central State Hospital, Petersburg, Va.

"Clinical Evaluation of Reserpine (Serpasil) in a State Hospital" - William L. Kirkpatrick and Foster Sanders, East Louisiana State Hospital, Jackson, La.

"Clinical and Psychological Observations on Psychiatric Patients Treated with Reserpine (Serpasil): A Preliminary Report" - Jay L. Hoffman and Leon Konchegul, Saint Elizabeths Hospital, Washington, D. C.

"Effects of Reserpine (Serpasil) on Central Autonomic Reactivity" - Robert Schneider, University of Oklahoma and Oklahoma Medical Research Foundation, Oklahoma City, Okla.

FRIDAY, FEBRUARY 4

Session Chairman: H. Hanson Twitchell
United States Air Force Surgeon General's Office, Washington, D. C.

9:30 A.M. -

"Clinical Use of Reserpine (Serpasil) in Psychiatry: Comparison with

Chlorpromazine" - M. Bleuler, University of Zurich, Zurich, Switzerland.

"Chlorpromazine (Thorazine) and Reserpine (Serpasil) in the Treatment of Psychoses" - Vernon Kinross-Wright, Baylor University College of Medicine, Houston, Texas.

"A Preliminary Report upon the Tranquilizing Effect of Reserpine (Serpasil)" J.C. Schraeder and R.R. Luttrell, Travis Air Force Base, Calif.

"Use of Reserpine (Serpasil) in Hyperactive and Irritable Infants" - Milton W. Talbot, Jr., Malone and Hogan Clinic-Hospital Foundation, Big Spring, Texas.

"The Use of Reserpine (Serpasil) in Office Psychiatry" - Frank R. Drake and F. G. Ebaugh, University of Colorado Medical School, Denver, Colo.

"The Use of Reserpine (Serpasil) in Private Psychiatric Practice" - Sydney Kinnear Smith, University of California, Berkeley, Calif.

Session Chairman: Harold E. Himwich
Galesburg State Research Hospital, Galesburg, Illinois

2:00 P.M. -

"Reserpine (Serpasil) as an Adjunct in the Therapy of Convulsive Neurological Disorders" - Vasilios S. Lambros, Arlington Hospital, Arlington County, Va.

"A New Approach to the Emergency Treatment of Sickness Caused by Narcotic Withdrawal" - Eugene F. Carey, Chicago Police Department, Chicago, Ill.

"The Tranquilizing Efficacy of Reserpine (Serpasil) in Dermatological Therapy" - Charles R. Rein, New York University-Bellevue Medical Center, New York, N. Y.

"Pre-operative Sedation with Reserpine (Serpasil)"- C. R. Stephen and Ruth C. Martin, Duke University School of Medicine, Durham, N. C.

"A New Adjunct in the Treatment of Resistant Headache Patterns" - B. M. Barrett, Pensacola, Fla., and F. K. Hansel, Washington University School of Medicine, Saint Louis, Mo.

"Use of Reserpine (Serpasil) as an Adjunct in Asthma and Emphysema Therapy" - Maurice S. Segal, Tufts College Medical School and Boston City Hospital, Boston, Mass.

"Treatment of Headache with Reserpine (Serpasil)" - Arnold F. Friedman, Montefiore Hospital, New York, N. Y.

BIOFLAVONOIDS AND THE CAPILLARY

Friday, February 11, 1955

Conference Co-Chairmen:
Gustav J. Martin, The National Drug Company, Philadelphia, Pa., and Albert Szent-Gyorgyi, Institute for Muscle Research, Woods Hole, Mass.

FRIDAY, FEBRUARY 11, 1955

LABORATORY SESSION

Session Chairman: Albert Szent-Gyorgyi
Institute for Muscle Research, Woods Hole, Mass.

9:00 A.M. -

Opening Remarks – Hilary Koprowski, Chairman, Section of Biology, The New York Academy of Sciences; Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

"Chemistry of the Bioflavonoids" - W. E. Baier, Sunkist Growers, Ontario, Calif.

"Biochemistry of the Bioflavonoids" – Gustav J. Martin, The National Drug Company, Philadelphia, Pa.

"Estrogenic Activity of Some Isoflavone Derivatives" – Edmund Cheng, Iowa Agricultural Experiment Station, Ames, Iowa,

"Biogenesis of the Bioflavonoids" – Franz Moewus, Columbia University, New York, N. Y.

"Physiology of the Capillary Bed" – Richard E. Lee, Cornell University Medical College, New York, N. Y.

"Structural Makeup of the Capillary Wall" – Benjamin Zweifach, New York University, Washington Square College, New York, N. Y.

"The Role of Flavonoids in Coumarin Anticoagulant Therapy" – Charles Brambel, University of Maryland; Director of Laboratories, Mercy Hospital, Baltimore, Md.

CLINICAL SESSION

Session Chairman: John B. Youmans
Vanderbilt University, School of Medicine, Nashville, Tenn.

2:00 P.M. -

"Bioflavonoids and Ascorbic Acid in Rheumatic Fever" – James F.

Rhinehart, University of California, San Francisco, Calif.

"Decidual Bleeding in Pregnancy" – Carl T. Javert, Cornell University Medical College, New York, N. Y.

"The Management of Habitual Abortion" – Robert B. Greenblatt, Medical College of Georgia; University Hospital, Augusta, Ga.

"A Rationale for the Use of Hesperidin and Ascorbic Acid in the Management of Poliomyelitis" – George J. Boines, Wilmington General Hospital, Wilmington, Del.

"Summary of the Clinical Aspects of Bioflavonoids and Ascorbic Acid" – John B. Youmans, Vanderbilt University, School of Medicine, Nashville, Tenn.

"Perspectives for the Bioflavonoids" – Albert Szent-Gyorgyi, Institute for Muscle Research, Woods Hole, Mass.

NEW MEMBERS

Elected February 24, 1955

LIFE MEMBERSHIP

Brody, Ormond Visor, Ph.D., Biophysical Chemistry. Research Fellow, Yale University, New Haven, Conn.

SUSTAINING MEMBERSHIP

Hardy, Thomas Edward, M.D., Medicine. Private Practice, Chicago, Ill.

Hellman, Emanuel, M.D., Cardiology. Director, Cardiac Clinic, Gouverneur Hospital, New York, N. Y.

May, Ben, Medical Research. New York, N. Y.

Vogel, Alfred M., Ph.D., Chemical Instrumentation. Chairman, Department, Chemistry, Adelphi College, Garden City, N. Y.

ACTIVE MEMBERSHIP

Adamsons, Karlis, Jr., M.D., Pharmacology. Research Fellow, National Foundation for Infantile Paralysis, New York, N. Y.

Altchek, Albert, M.D., Medicine. Chief Resident, Mt. Sinai Hospital, New York, N. Y.

Bachtold, John G., Ph.D., Virology. Assistant Research Professor, University of Utah, Salt Lake City, Utah.

Badollet, M.S., Mining. Section Chief, Asbestos Research, Johns Manville Research Center, Manville, N. J.

Bailey, Charles A., M.D., Infectious Disease. Private Practice, Ridgewood, N. J.

Bakst, Hyman, M.D., Internal Medicine. Beth Israel Hospital, New York, N. Y.

Baum, Samuel Meyerson, M.D., Biology. Associate Radiologist, City Hospital, New York, N. Y.

Beber, Charles R., M.D., Biological Sciences. Intern, Mt. Sinai Hospital, New York, N. Y.

Benham, G. H., Ph.D., Biochemistry. Supervisor, Armour Research Foundation, Chicago, Ill.

Berkett, George D. B., M.D., Orthopedic Surgery. Assistant Professor, Tulane University, New Orleans, La.

Bhargava, Krishna P., M.D., Pharmacology. Associate Professor, Pharmacology, University of Lucknow, Lucknow, India.

Bianchi, Albert V., B.S., Histology. Technician, New York University, New York, N. Y.

Bock, Lux H., M.D., Medicine. Private Practice, St. Louis, Mo.

Bonnafous, Yvonne, B.S., Parasitology. Research Assistant, American Museum of Natural History, New York, N. Y.

Bouchard, Edward F., Medicinal Chemistry. Manager, Pharmaceutical Section, Technical Service Department, Chas. Pfizer and Company, Brooklyn, N. Y.

Brandner, Bernard, M.D., Medicine. Private Practice, Brooklyn, N. Y.

Brazeau, Paul, Ph.D., Renal Physiology. Associate in Pharmacology, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Bruner, Richard C., B.S., Biological Sciences. Head, Pharmaceutical Division, Sunkist Growers, Ontario, Calif.

Caliva, Francis S., M.D., Internal Medicine. Assistant Professor, Medicine, Syracuse, N. Y.

Carey, Richard A., M.D., Endocrinology. Medical Technical Director, Fine Chemicals Division, American Cyanamid Company, New York, N. Y.

Carleton, Richard O., B.S., Biochemistry. Research Chemist, Mound Laboratory, Monsanto Chemical Company, Miamisburg, Ohio.

Chakravarty, Nirmal K., M.D., Pharmacology. Research Fellow, University of Utah, Salt Lake City, Utah.

Chao, Jowett, Ph.D., Entomology. Assistant Research Zoologist, University of California, Los Angeles, Calif.

Chapin, Horace Beecher, M.D., Biology. Clinical Assistant, Roosevelt Hospital, New York, N. Y.

Clemedson, Carl-Johan, M.D., Medicine. Chief, Medical Department, Research Institute of National Defense, Sundbyberg, Sweden.

Clemens, Ted, Jr., M.D., Internal Medicine. Resident, University Hospitals, Oklahoma City, Okla.

Cooley, Donald G., Medicine. Free-Lance Science Writer, New York, N. Y.

Crawford, Lloyd, M.D., Pediatric Allergy. Assistant in Pediatrics, University of Tennessee, Memphis, Tenn.

Desantis, Dahlia, M.A., Biology. Science Instructor, Mary Immaculate Hospital, School of Nursing, Jamaica, N. Y.

Doull, John, Ph.D., Pharmacology. Assistant Professor, Department, Pharmacology, U.S.A.F. Radiation Laboratories, University of Chicago, Chicago, Ill.

Downey, Helen V., Ph.D., Physiology. Assistant Professor, Hunter College, New York, N. Y.

Duane, Rose T., B.A., Immunohematology. Senior Medical Technician, University of California, Medical Center, San Francisco, Calif.

Eisenstein, Victor W., M.D., Psychiatry. Chief, Psychiatry Clinic, Lenox Hill Hospital, New York, N. Y.

Fellowes, Oliver N., Ph.D., Virology. Virologist, Plum Island Animal Disease Laboratory, U.S. Department of Agriculture, Greenport, N. Y.

Ferno, Ove, Organic Chemistry. Director, Research, Aktiebolaget Leo, Hälsingborg, Sweden,

Fischer, David S., B.A., Clinical Medicine. Medical Student, Harvard Medical School, Boston, Mass.

Flores, Jose, M.D., Internal Medicine. Private Practice, Managua, Nicaragua.

Florey, M. Ethel, M.D., Antibiotics. Medical Research Worker, Sir Wm. Dunn School of Pathology, Oxford, England.

Foster, Henry L., D.V.M., Laboratory Animals. President, Director, Charles River Breeding Laboratories Inc., Wilmington, Mass.

Fox, Lewis, D.D.S., Dentistry. Private Practice, South Norwalk, Conn.

Frank, George B., M.S., Neurophysiology. Graduate Student, McGill University, Montreal, P.Q., Canada.

Franklin, Homer H., B.A., Physiology. Director, Professional Service, The Harrower Laboratory Inc., Jersey City, N. J.

Frederick, Jerome F., M.Sc., Enzymes. Research Director, Dodge Chemical Company, New York, N. Y.

Frohman, I. Phillips, M.D., Medicine. Private Practice, Washington, D. C.

Gajewski, John E., Ph.D., Clinical Therapeutics. Associate, Department, Clinical Investigation, Parke, Davis and Company, Detroit, Mich.

Gamba, Joseph, M.D., Biology. Private Practice, Newark, N. J.

Gambescia, Joseph M., M.D., Internal Medicine. Assistant Professor, Department Gastroenterology, Hahnemann Hospital, Philadelphia, Pa.

Garber, Jared Y., M.D., Medicine. Private Practice, Lake Charles, La.

Garol, Hugh William, M.D., Neurology. Neurosurgeon, San Francisco, Calif.

Gaylord, William H., Ph.D., Virology. Instructor, Yale University, New Haven, Conn.

Genkins, M. H., M.D., Surgery. Private Practice, Norristown, Pa.

Gerbasi, Francis S., M.D., Surgery. U.S.N.R., La Mesa, Calif.

Gerone, Peter J., D.Sc., Virology. Bacteriologist, U.S. Army Chemical Corps., Frederick, Md.

Gerstle, M., M.D., Pathology. Private Practice, New York, N. Y.

Geyer, Hal W., M.D., Psychiatry. Private Practice, Milford, Del.

Gilroy, Francis J., M.D., Internal Medicine. Private Practice, Cliffside Park, N. J.

Gingras, Nap J., M.D., Medicine. Private Practice, Augusta, Me.

Ginsberg, Leon, M.D., Neuropsychiatry. Director, Essex County Mental Hygiene Clinics, Cedar Grove, N. J.

Gislason, Gerhard John, M.D., Medicine. Assistant Professor, Urology, Hahnemann Medical College, Philadelphia, Pa.

Givner, Isadore, M.D., Ophthalmology. Private Practice, New York, N. Y.

Gluck, Edward J., M.D., Internal Medicine. Senior Resident, Medicine, Beth Israel Hospital, New York, N. Y.

Goldman, Max, M.D., Psychiatry. Private Practice, Boston, Mass.
Goldstein, Nathan A., M.D., Medicine. Private Practice, Flushing, N. Y.
Goldstein, Oscar E., M.D., Medicine. Instructor in Medicine, Cornell University, New York, N. Y.
Gonzalez, Carlos, M.D., Medicine. Private Practice, Rio Piedras, Puerto Rico.
Gorman, Mike, M.A., Psychiatry. Executive Director, National Mental Health Committee, Washington, D.C.
Grauer, Robert C., M.D., Endocrinology. Director, Laboratory, Singer Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.
Greenberg, Morris Z., M.D., Obstetrics. Private Practice, Detroit, Mich.
Greenblatt, R. B., M.D., Endocrinology. Professor, Endocrinology, University of Georgia, Augusta, Ga.
Greene, William Leonard, M.D., Medicine. Private Practice, Beaumont, Texas.
Griffen, Joseph A., M.D., Medicine. Assistant Physician, Brigham Hall Hospital, Canandaigua, N. Y.
Groel, John T., M.D., Chemotherapy of Infectious Diseases. Associate Medical Director, Squibb Institute for Medical Research, Short Hills, N. J.
Gruen, Arno, Ph.D., Psychology. Instructor, Queens College, Flushing, N. Y.
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